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Award Number: DAMD17-01-1-0333

TITLE: Summer Undergraduate Breast Cancer Research Program

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Linda Blockus, Ph.D.

CONTRACTING ORGANIZATION: University of Missouri-Columbia
Columbia, Missouri 65211

REPORT DATE: June 2004

TYPE OF REPORT: Annual Summary

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	June 2004	Annual Summary (15 May 2001 - 14 May 2004)

4. TITLE AND SUBTITLE
Summer Undergraduate Breast Cancer Research Program

5. FUNDING NUMBERS
DAMD17-01-1-0333

6. AUTHOR(S)
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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. Abstract *(Maximum 200 Words)* *(abstract should contain no proprietary or confidential information)*
The Summer Undergraduate Breast Cancer Research Program (SUBCRP) at the Univ. of Missouri-Columbia (MU) supported 4 students in 2003.These students participated in faculty-mentored research projects for eight weeks and participated in seminars, brown-bag lunches, and specialty discussions on research, clinical trials, career opportunities, preparing for graduate school, and ethics. The 4 SUBCRP students joined the activities of the MU's Life Sciences Undergraduate Research Opportunities Program, including 80 other students involved in a wide variety of research experiences. The SUBCRP students included two female African Americans and two female Caucasian students. Faculty from Biochemistry, Biological Sciences, Family & Community Medicine, Molecular Microbiology & Immunology, and served as mentors. Research projects included: 1) The difference in age at menarche observed between African American and Caucasian athletes as it relates to breast cancer risk; 2) The effect of MIP-α on cancer cell differentiation; 3) Characterization of Glc7 suppressors and 4) sAPPα is released following activation of the $P2X_7$ recptor in 1321N1 astrocytoma cells. Seven other summer interns participated in cancer related research in 2003.

14. SUBJECT TERMS
breast cancer, undergraduate, research, training

15. NUMBER OF PAGES
105

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

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INTRODUCTION

The Summer Undergraduate Breast Cancer Research Program (SUBCRP) at the University of Missouri-Columbia (MU) supported four students in 2003. These students participated in faculty-mentored research projects for eight weeks and participated in seminars, brown-bag lunches, and specialty discussions on research, clinical trials, career opportunities, preparing for graduate school, communication skills, and ethics. The four SUBCRP students joined the activities of the University's Life Sciences Undergraduate Research Opportunity Program, including over 45 students from across the country involved in a wide variety of research experiences. Seven of these students also conducted cancer-related research projects; however, received support from five other funding sources.

BODY

Recruitment and student selection

Information on the 2003 summer program was mailed to biology departments in Missouri and surrounding states, as well as other institutions that have a summer intern partnership with MU. Student applicants were asked to provide a transcript, personal statement, resume and letter of recommendation. Applications were reviewed by a four person committee and then top applications were discussed by the Project Director and Program Coordinator for final selection/mentor placement. University of Missouri-Columbia students applied for funding through the Life Sciences Undergraduate Research Opportunity Program (LS UROP). Their application included a transcript, two letters of recommendation, personal statement and a project proposal prepared with the guidance of their faculty mentor. MU students were reviewed by faculty members of the LS UROP Advisory Committee.

A total of four female students were selected and funded by this grant in 2003, including two African American women. These students worked with four faculty members from the departments of Biochemistry, Biological Sciences, Family & Community Medicine, and Molecular Microbiology & Immunology. The seven other students also conducting cancer research (funded from other sources) included two African American students and one Hispanic student.

Research projects

Students worked in their research labs on a full-time basis for 8 weeks (June 9 - August 1, 2003) and received a stipend scholarship. MU students received an amount consistent with the other LS UROP interns ($2600). Non-MU students received additional funding to off-set their living expenses. The student interns participated in weekly lab group meetings with their faculty mentor and other lab team members. On July 31, 2003, the 4 students funded by this grant participated in the Fourteenth Annual MU Undergraduate Research Science Symposium and Luncheon. These students, along with over 80 other summer science interns, displayed posters describing their research projects, and received certificates at the awards luncheon. Invited guests included faculty mentors, lab members, campus administration, and the local media. Complete descriptions of the 2003 projects can be found in the abstract booklet for the Undergraduate Research Science Symposium. A list of the 2003 students funded on this grant, their home institutions, majors, hometowns, faculty mentors (and academic departments), and the research topics appear below.

Nicole Campbell, University of Missouri-Columbia, Biology major from St. Louis, Missouri
Mentor: Dr. Shanna Swan, Family & Community Medicine
The difference in age at menarche observed between African American and Caucasian athletes as it relates to breast cancer risk

Erin Cazel, Stanford University, Biological Sciences major from Palo Alto, California
Mentor: Dr. Thomas Phillips, Biological Sciences
The effect of MIP-α on cancer cell differentiation

Mary Millwood, Jacksonville State University, Biochemistry major from Moulton, Alabama
Mentor: Dr. John Cannon , Molecular Microbiology & Immunology
Characterization of Glc7 suppressors

LaTasha A. Rabsatt, Prairie View A&M University, Biology major from Killeen, Texas
Mentor: Dr. Gary Weisman, Biochemistry
sAPPα is released following activation of the $P2X_7$ recptor in 1321N1 astrocytoma cells

Seven other summer interns participated in cancer related research in 2003:

Leilani Castleman, University of Missouri-Columbia, Biology major from Houston, Missouri
Mentor: Dr. Dennis Lubahn, Biochemistry/Child Health
Searching for new ERRβ isoforms in the mouse model
Funded by the NSF Louis Stokes Missouri Alliance for Minority Participation

Alexis Cody, University of Missouri-Columbia, Biology and Psychology major from Jefferson City, Missouri
Mentor: Dr. Dennis Lubahn, Biochemistry/Child Health
Phytoestrogen regulation of phase II enzymes in prostate cancer cell lines
Funded by the NSF Louis Stokes Missouri Alliance for Minority Participation

Maggie Christ, University of Missouri-Columbia, Biology major from Imperial, Missouri
Mentor: Dr. Mark Kirk, Biological Sciences
Selective migration of neutralized mouse embryonic stem cells towards tumor cells and conditioned media
Funded by the Arts and Science Undergraduate Research Mentor Program

Shorouk Dannon, University of Missouri-Columbia, Chemistry major from St. Charles, Missouri
Mentor: Dr. Silvia Jurisson, Chemistry
Synthesis of model Re(v) and Re(III) Schiff Base radiopharmaceutical complexes
Funded by the Stevens' Fellowship Summer Research Program

Stephanie Lane, University of Missouri-Columbia, Chemistry major from Springfield, Missouri
Mentor: Dr. Silvia Jurisson, Chemistry
Radiopharmaceutical research: Coordination of Re to tetradentate ligands
Funded by the Arts and Science Undergraduate Research Mentor Program

Holly Powell, University of Missouri-Columbia, Biochemistry major from St. Peters, Missouri
Mentor: Dr. Thomas Quinn, Biochemistry
Development of a potential imaging and radiotherapeutic agent for breast cancer: ^{111}In-DOTA-GA-KCCYSL
Funded by the Life Sciences Undergraduate Research Opportunity Program

Richard Steward III, University of Arkansas-Pine Bluff, Biology Major from Pine Bluff, Arkansas
Mentor: Dr. Dennis Lubahn, Biochemistry/Child Health
Is estrogen regulation of cancer protected genes dependent on NRF2?
Funded by NSF Research Experiences for Undergraduates

Educational/Career Activities and Workshops

In addition to their research projects, interns participated in a variety of enrichment and social activities as part of the summer undergraduate research community at MU. The activities were organized and hosted by Dr. Joel Maruniak, Associate Professor of Biological Sciences, and Program Coordinator Dr. Linda Blockus. In addition to the 4 Breast Cancer interns, we had 20 MU students supported on university funds and foundation grants, 13 students participating in the Plant Genomics Internship Program, and 10 NSF-REU interns that were all regular participants in the educational and career activities. Our activities for 2003 included:

* Staff from the campus Environmental Health and Safety Office presented special workshops on lab safety, hazardous materials, and radioactive materials.

* Non-MU students were given a special tour of the main library by a science reference librarian.

* Three brown bag lunches provided an informal opportunity for students to present their projects in small groups to other life sciences interns.

* Dr. Joel Maruniak presented a brown bag lunch workshop on 'Preparing your Abstract and Research Poster' in preparation for the Undergraduate Research Science Symposium.

* Dr. Karen Cone, MU Division of Biological Sciences and Missouri Maize Project, presented 'Scientific essentials for research students.'

Evening seminars and brown bag lunches related to career decisions and issues included:

* 'Finding your right livelihood' by Dr. Joel Maruniak, MU Division of Biological Sciences.

* 'Balancing a family and a career in science' by Dr. David Setzer, MU Division of Biological Sciences and Dr. Linda Headrick, MU School of Medicine and Senior Associate Dean.

* 'Applying to graduate programs in the life sciences' by Dr. Mannie Liscum, Biological Sciences and Dr. David Emerich, Biochemistry.

* 'MD, PhD, or Both?' by MD/PhD student Adam Jackson.

* 'Administering science research' by Dr. James Coleman, MU Vice Provost for Research.

* 'Being a faculty member at a small college' by Dr. Michael Torres, Maryville College.

* 'Research in Industry' by Dr. Noel Premkumar, ABC Laboratories.

Evening seminars and brown bag lunches related to recent scientific discoveries and their social implications included:

* 'Cloning pigs: A story of science and bioethics' by Dr. Randall Prather, MU Department of Animal Sciences.

* 'Protection of biodiversity in riparian habitats: Criteria for buffers and core habitat of amphibians and reptiles' by Dr. Ray Semlistch, MU Division of Biological Sciences.

* 'The science of telomeres and life extension: The fountain of youth?' by Dr. Joel Maruniak, MU Division of Biological Sciences.

* 'Stem cell biology' by graduate student Jason Meyer.

* 'Endocrine disruptors: Science research and public policy' by Dr. Fred vom Saal, MU Division of Biological Sciences.

Specialty discussions were held specifically for students in the Breast Cancer Summer Internship Program and the Plant Genomics Internship program; however, all students were encouraged to attend:

* 'A comparative oncology approach to breast cancer research' by Dr. Carolyn Henry, MU Department of Veterinary Medicine & Surgery.

* 'Clinic research trials for breast cancer' by Dr. Lisa Jacobs, MU Department of Surgical Oncology.

* 'Cancer treatment survivorship issues: Post-breast cancer lymphedema' by Dr. Jane Armer, Director of Nursing Research, Ellis Fischel Cancer Center.

* 'Biomedical applications of radioisotopes' by Dr. Susan Lever, MU Department of Chemistry and Nuclear Research Reactor.

* 'An integrated genetic and physical map for maize' by Dr. Jack Gardiner, Missouri Maize Mapping Project.

* 'Genetic engineering in agriculture: The GMO debate' by Dr. Georgia Davis, MU Department of Agronomy, and Dr. Karen Cone, MU Division of Biological Sciences.

* 'Functional genomics' by Dr. Gary Stacey, MU Department of Plant Microbiology and Pathology.

A field trip to the Donald Danforth Plant Sciences Center and the Monsanto Plant and Biotechnology campus (both in St. Louis) was offered to summer interns. Approximately 20 students attended. Students also had the option to participate in CPR Training and Basic First Aid courses arranged for our programs by the MU Office of Environmental Health and Safety. About 20 students received certification. The annual intern/mentor BBQ was held early in the summer so that students could get to know their mentors and their families on a more informal basis.

Assessment and Evaluation

Summer interns and faculty participated in the on-going efforts of the LS UROP office to determine the impact of summer research internships and activities and to improve faculty mentoring skills. Students were asked to complete two "critical incident reports" to provide insight into what events during the program have been most important to their consideration of a career in science. Coding of the open-ended responses indicates that there are both negative and positive events, and that speakers and the poster session play an important role along with the actual laboratory experience. Data is currently being analyzed to determine if pre-graduate students differ from pre-medical students in the types of events that have the most impact. Summer interns are asked to complete the "Confidence in Inquiry-related Skills" survey at the beginning and end of the summer program. In addition to comparing the pre/post scores of intern confidence on 20 scientific research skills, intern scores are also compared with their faculty mentor's assessment for each skill at the end of the program. Surveys administered at the end of the summer to interns and faculty mentors request information on the quality, quantity, content and method of communication that the student has with the mentor and others in his/her lab. This data will be linked to items in the "Confidence in Inquiry-related Skills" and used to provide faculty mentors as a group with feedback. LS UROP also maintains a student database of previous interns (currently over 1000 students since 1989) with educational and career information for longitudinal tracking. The database also contains information on student publications and poster presentations. Alumni are contacted periodically to update their file with graduate degrees earned and career information.

KEY RESEARCH ACCOMPLISHMENTS

The primary purpose of this project is to provide a research experience for undergraduates. As such, any significant results of their research projects would be incorporated as preliminary data into the on-going activities of their faculty mentor's laboratory.

REPORTABLE OUTCOMES

1) Data collected from students and faculty as part of the larger LS UROP assessment and evaluation project is still being analyzed for results and eventual publication.

2) We will be contacting Breast Cancer interns to determine if they have completed their bachelor's degree, entered graduate/professional school, and co-authored any additional publications or presentations.

3) Two 2003 breast cancer interns (Nicole Campbell and LaTasha Rabsatt) attended the Annual Biomedical Research Conference for Minority Students (ABRCMS) in San Diego in October 2004. Nicole Campbell presented her research poster at this conference. Alexis Cody and Richard Steward, who also did cancer projects but were funded from other sources, presented their work at the conference as well.

CONCLUSIONS

We continue to be pleased with the student participation and faculty mentoring for the Summer Undergraduate Breast Cancer Research Program. Because of the increased visibility of breast cancer undergraduate research opportunities on campus due to the U.S. Army program, additional students are conducting cancer-related research and are being funded through other sources.

REFERENCES

None.

APPENDICES

One copy of the abstract book for the 14th Annual Undergraduate Research Science Symposium has been sent along with this annual report.

14th Annual

Undergraduate Research Science Symposium

University of Missouri ~ Columbia

1:30 ~ 3:30 p.m.

Thursday, July 31, 2003

Donald W. Reynolds Alumni Center

University of Missouri - Columb

Fourteenth Annual Undergraduate Research Science Symposium

Thursday, July 31, 2003
Donald W. Reynolds Alumni Center
1:30 - 3:30 p.m.

Abstracts in this book describe the scientific research projects of over eighty students in summer research internship programs sponsored by the University of Missouri - Columbia.

Participating Research Departments and Divisions

Agronomy
Animal Sciences
Anthropology
Biochemistry
Biological Engineering
Biological Sciences
Cardiology
Chemistry
Child Health
Dalton Cardiovascular Research Center
Educational & Counseling Psychology
Electrical Engineering
Family & Community Medicine
Mathematics
Medical Pharmacology & Physiology
Molecular Microbiology & Immunology
Nutritional Sciences
Plant Microbiology & Pathology
Political Science
Psychology
Sociology
Veterinary Biomedical Sciences

Abstract Book Prepared by:
LS UROP Office
422 Tucker Hall
University of Missouri - Columbia
Columbia, MO 65201-7400
(573) 882-5979
www.lsurop.missouri.edu

Symposium Web Site and On-Line Abstracts Created by Ramona Fairchild and Alan Marshall, Division of Biological Sciences.

Participants and Sponsors

The Summer 2003 Undergraduate Research Science Symposium represents the fourteenth year of summer research intern presentations at the University of Missouri-Columbia. Student interns displaying their research projects at the Symposium are supported by one of eight formal summer programs or are funded individually through their faculty mentor or other on-going program. Undergraduates hail from over thirty-five institutions of higher education and their faculty mentors come from over twenty academic units at MU.

Participating programs include the MU Life Sciences Undergraduate Research Opportunity Program (LS UROP), the Life Sciences National Science Foundation Research Experiences for Undergraduates (NSF-REU) Program, the Plant Genomics Internship at MU (PGI@MU), the Summer Undergraduate Breast Cancer Research Program (SUBCRP), the MU Monsanto Undergraduate Research Fellowship (MURF), the Louis Stokes Missouri Alliance for Minority Participation, the Summer Pre-Graduate Research Experience for Students in the Humanities, and the Missouri Ozark Forest Ecosystem Project (MOFEP) Internships in Avian Ecology.

Life Sciences Undergraduate Research Opportunities Program (LS UROP)

The Summer 2003 LS UROP Undergraduate Research Internships are funded by the MU Office of the Provost/Life Science Mission Enhancement. MU students conducted an independent project in the life sciences under the guidance of an MU faculty member to experience research first-hand and prepare for biomedical, scientific and teaching careers. Students attend evening seminars and brown bag lunches to discuss issues related to scientific research, including ethics, career paths, emerging research areas, and opportunities for post-baccalaureate study. This program is coordinated by the LS UROP office.

2002-2003 LS UROP Advisory Board:

Dr. Sandra Abell, Science Education
Dr. Karen Bennett, Molecular Microbiology and Immunology
Dr. Karen Cone, Biological Sciences
Dr. John David, Biological Sciences
Dr. Kevin Fritsche, Animal Sciences
Dr. Joe Polacco, Biochemistry
Dr. Ray Semlitsch, Biological Sciences
Dr. James Spain, College of Agriculture, Food & Natural Resources
Dr. Roger Sunde, Nutritional Sciences
Dr. Henry White, Physics and Astronomy
Dr. Warren Zahler, Biochemistry

Program Coordinator:	Dr. Linda Blockus
Program Assistant:	Susan Renoe
Staff Assistants:	Megan Odneal, Suzy Otto

National Science Foundation - Research Experience for Undergraduates (Life Sciences)

Currently in the first year of a new five year grant from NSF, this program supports undergraduates from regional and minority serving institutions to conduct independent research projects with faculty at MU. Students selected by the cooperating institutions participate in an eight week summer research program in the areas of cell, molecular, and genetic biology. Students attend educational enrichment activities with the LS UROP Interns throughout the summer. Continuing partnerships have been established with Barry University, The College of St. Elizabeth, Florida A&M University, Grinnell College, Long Island University-Brooklyn Campus, Medgar Evers College, Prairie View A&M University, Southwest Missouri State University, Truman State University, University of Arkansas-Pine Bluff, and Xavier University of Louisiana. The Division of Biological Sciences and LS UROP have received continuous support from the NSF-REU program since 1991.

Principle Investigator:	Dr. John David, Biological Sciences
Co-PI:	Dr. Linda Blockus, LS UROP

Plant Genomics Internships at MU

MU has long been nationally recognized as a center for plant genetics research. The purpose of the Plant Genomics Internships at MU (PGI@MU) is to demonstrate the excitement and career options available in the field of plant genomics research to talented undergraduates. Fifteen faculty from seven research units, representing ten projects funded through the NSF Plant Genome Research Program serve as mentors for this program. In addition to attending evening seminars and brown bag lunches with the other summer interns, PGI students participated in three specialty presentations on plant genomic topics and toured the Danforth Plant Center and the plant and biotechnology research facilities at Monsanto. The MU plant genomic faculty, in collaboration with LS UROP, have secured a three-year, $191,000 grant from the National Science Foundation to fund this summer program.

Program Director: Dr. Karen Cone, Biological Sciences

Summer Undergraduate Breast Cancer Research Program

Over twenty-five externally funded breast-cancer research projects are currently underway under the guidance of MU faculty in five colleges and schools. The Summer Undergraduate Breast Cancer Research Program provides a faculty mentored research internship experience related to breast cancer. In addition to evening seminars and brown bag lunches with other summer interns, breast cancer interns participated in specialty presentations on clinical research. Support for this program comes from a three-year, $129,500 competitive grant awarded to MU specifically for undergraduate research training from the U.S. Army Medical Research and Material Command. This program is coordinated by the LS UROP office.

Principle Investigator: Dr. William Folk, Associate Dean for Research, School of Medicine

Co-PI: Dr. Linda Blockus, LS UROP

MU Monsanto Undergraduate Research Fellowship

The Interdisciplinary Plant Group (IPG), in conjunction with the Life Sciences Undergraduate Research Opportunity Program coordinates funding for undergraduate research in the area of plant biology, proteomics, and bioinformatics. This project is funded by a four year grant to MU from the Monsanto Life Sciences Company. MURF students participate in all LS UROP sponsored educational programs.

Principle Investigators: Dr. Doug Randall, Biochemistry
Dr. John Walker, Biological Sciences

Missouri Ozark Forest Ecosystem Project - Internships in Avian Ecology

The Missouri Ozark Forest Ecosystem Project (MOFEP) is a multi-dimensional experiment examining the effects of two timber harvest techniques on the Ozark ecosystem. This year was the seventh following timber removal, which was done in 1996. Funding for the avian research component comes from the Missouri Department of Conservation. Sixteen interns from across the U.S. and Mexico spent 10 weeks working in the Missouri Ozarks, with early efforts spent spot-mapping bird locations and finding and monitoring bird nests. Since late June, interns have been mist-netting and banding birds and working on individual research projects. Individual projects shown at the poster session involve all aspects of Ozark ecology and are completed while the students are still doing most of their normal duties for MOFEP.

Program Directors: Dr. John Faaborg, Biological Sciences, MU
Dr. Paul Porneluzi, Central Methodist College
Richard L. Clawson, Missouri Department of Conservation

The Louis Stokes Missouri Alliance from Minority Participation (LS-MOAMP)

LS-MOAMP in conjunction with the MU Graduate School offers talented underrepresented undergraduate collegians a bridge to graduate school. Providing additional financial, educational, and career development, the LS-MOAMP Summer Research Internship Program in Sciences, Technology, Engineering & Mathematics (STEM) assists them with successfully entering and completing graduate degree programs. By participating in the LS-MOAMP Summer Research Internship Program in STEM, collegians are provided with eight weeks of research experience under the direction of faculty mentors, with course credit and workshops to prepare them to obtain an advanced degree.

Principal Investigator:	Dr. Charles Sampson, Associate Vice Provost & Director of the Missouri Alliance for Minority Participation
Program Director:	Stephanie White Thorn, Director of Graduate Student Affairs
Administrative Assistant:	Charity Ogunbo
Graduate Assistant:	Jami P. Joyner

The Summer Pre-Graduate Research Experience for Students in the Humanities

The Graduate School at the University of Missouri-Columbia is proud to present a pilot program for undergraduate students across the nation. The Summer Pre-Graduate Research Experience for students in the Humanities (Summer Pre-Grad) offers undergraduate students the opportunity to work full-time on a research project under the direction of a MU faculty member. The program is designed for students sincerely interested in pursuing the graduate studies, preferably the Ph.D. degree. It is our sincere intent that this program serve to introduce students to the challenges, rigors and rewards of an academic life.

Principal Investigator:	Dr. Suzanne T. Ortega, Vice-Provost for Advanced Studies and Dean of the Graduate School
Coordinator:	Stephanie White Thorn
Program Assistant:	Charity Ogunbo
Graduate Assistant:	Jami P. Joyner

Students Participating in the Undergraduate Research Science Symposium July 31, 2003

Life Sciences Undergraduate Research Opportunities Program (LS UROP)

Adam Alter, University of Missouri - Columbia
Chris Blanner, University of Missouri - Columbia
James Bosanquet, University of Missouri - Columbia
Katie Connolly, University of Missouri - Columbia
Jason Gentry, University of Missouri - Columbia
Jeffrey LaCroix, University of Missouri - Columbia
Georgia Marsh, University of Missouri - Columbia
Andrea Miller, University of Missouri - Columbia
Andrew Mitchell, University of Missouri - Columbia
Darah Oxford, University of Missouri - Columbia
Jonathan Oxford, University of Missouri - Columbia
Holly Powell, University of Missouri - Columbia
Vinay Rawlani, University of Missouri - Columbia
Kimberly Willer, University of Missouri - Columbia
Rachel Williams, University of Missouri - Columbia
David Wirth, University of Missouri - Columbia
Christopher Yee, University of Missouri - Columbia

National Science Foundation - Research Experience for Undergraduates (Life Sciences)

William Alexander, Truman State University
Jonven Attia, Kingsborough Community College
Denise Cafiero, College of St. Elizabeth
Amy McCroskey, Southwest Missouri State University
Kyle Shull, Southwest Missouri State University
Tatiana Sousa, College of St. Elizabeth
Richard Steward, University of Arkansas-Pine Bluff
Monica Stutz, Truman State University
Gesulla Toussaint, Barry University
Jaqueline Weiss, Truman State University

Plant Genomics Internships at MU

Ashley Baker, Oklahoma State University
Britton Boyd, Mississippi State University
Kimberly Crouch, University of Missouri - Columbia
Emily Dunn, Truman State University
Mark Ebel, University of Missouri - Rolla
Melanie Evans, University of Missouri - Columbia
Mark Goebel, University of Illinois
Kate Hart, University of Missouri - Columbia
Henrick Horita, University of Washington
Rachel Maltman, University of Missouri - Columbia
Barbara Sanchez-Neri, Purdue University
Mauricia Victor, Medgar Evers College
April Wynn, McMurray University

MU Monsanto Undergraduate Research Fellowships

Dana Buder, University of Missouri - Columbia
Christopher Durant, University of Missouri - Columbia
Sarah Youngstrom, University of Missouri - Columbia

Summer Undergraduate Breast Cancer Research Program

Nicole Campbell, University of Missouri - Columbia
Erin Cazel, Stanford University
Mary Millwood, Jacksonville State University
LaTasha Rabsatt, Prairie View A&M University

Missouri Ozark Forest Ecosystem Project - Internships in Avian Ecology

Rebekah Augustine, University of Delaware
William S. Beatty, University of Missouri - Columbia
Justan Blair, Central Methodist College
Christy Bowersox, Lycoming College
Federico A. Enriquez, Instituto Tecnologico de Cd. Victoria
Yasmin Ali Garcia Carbonell, Instuto Tecnologico de Cd. Victoria
Merande M. Green, Truman State University
Paul Hage, University of Missouri - Columbia
Scott Loss, University of Wisconsin - Stevens Point
Matthew C. Miller, Earlham College
Joseph Patson, University of Delaware
Matthew E. Rice, Slippery Rock University
Annie Silkowski, University of Connecticut
Patrick D. Stanley, University of Missouri - Columbia
Abigail B. Walker, Springfield College
Gregory T. Wann, University of Missouri - Columbia

Louis Stokes Missouri Alliance for Minority Participation

Barbara Alcocer, Universidad Del Turabo
LaWanda Barnes, Fisk University
Leilani Castleman, University of Missouri - Columbia
Alexis Cody, University of Missouri - Columbia
Ivy Huntley, Dillard University
Maricruz Pajares, College of St. Elizabeth
Jeremy Raincrow, University of Central Oklahoma
Khandicia Randolph, University of Missouri - Columbia
Debora Rivera, College of St. Elizabeth
Aida Ruiz, Universidad Del Turabo
Jasmine Scott, Alcorn State University
Marcos Searight, Jackson State University
Jennifer Stewart, University of Missouri - Columbia
Eugene Walton, Jr., Truman State University

Summer Pre-Graduate Research Experience for Students in the Humanities

Rhea Hayden, University of Northern Iowa
Miriam Warren, Mills College

Students Funded by Other Sources

Maggie Christ, University of Missouri - Columbia, A&S Undergraduate Research Mentor Program
Shorouk F. Dannoon, University of Missouri - Columbia, Stevens' Fellowship Summer Research Program
Mike Gerau, Universiry of Missouri - Columbia, NSF Plant Genome Research Prgoram Grant to G. Davis
Stephanie Lane, University of Missouri - Columbia, A&S Undergraduate Research Mentor Program
Ming Hui Lin, University of Missouri - Columbia, NIH Grant to G. Sun
Scott Schoenleber, University of Missouri - Columbia, A&S Undergraduate Reseach Mentor Program
Antonia Sisneros, University of Missouri - Columbia, McNair Scholars Program
Melissa Steward, University of Missouri - Columbia, Academic Year LS UROP
Kevin Tan, Cornell University, NIH Grant to G. Sun
Sathya Vadivelu, University of Missouri - Columbia, University of Missouri Research Board Grant to M. Kirk

Barbara E. Alcocer

Hometown: Caguas, Puerto Rico

Major: Chemistry

University: Universidad del Turabo

Faculty Mentor: Dr. Paul Duval, Chemistry

Funded by Louis Stokes Missouri Alliance for Minority Participation

Uranium: Behaviors in the environment

Barbara E. Alcocer Leon and Paul Duval

The dominant feature of the actinides is their nuclear instability, as manifest in their radioactivity. Uranium is a hard, dense, malleable, ductile, silver-white, radioactive metal of the actinide series. It is one of the two only actinides found in the earth's crust in appreciable quantities. It is hazardous to the environment, because it affects a person's long term health. This mineral consists of the combination of two isotopes: 99% U-238 and 1% U-235. It is utilized in nuclear reactors and nuclear weapons. Uranium has a large density and like most metal extractions, a number of methods can be used. The cheapest extraction method is mined uranium IV oxide (UO_2) which is found in the environment. This research helps us to determine the existence of multiple behaviors of UO_2 using different techniques. In this study, we looked for given products solubility in various aqueous media, new separation techniques for processing and remediation, demonstrated if an element is substituted by another one for oxidation/reduction (redox), manipulated air-sensitive compounds under inert conditions using dual vacuum/Schlenk lines and the glovebox system. In all experiments performed, the product's components were successfully determined.

William G. Alexander

Hometown: Salem, Missouri

Major: Biology

University: Truman State University

Faculty Mentor: Dr. Jan A. Miernyk, Biochemistry

Funded by National Science Foundation - REU (Life Sciences)

atDjB48, a previously uncharacterized molecular chaperone from *Arabidopsis thaliana*

William G. Alexander, Kent Strodtman, and Jan A. Miernyk

DnaJ was originally isolated from *Escherichia coli* as a 41 kDa heat shock protein. Subsequently, DnaJ was found to interact with DnaK and GrpE, forming a multi-component molecular chaperone machine. DnaJ functions in this machine by binding to the ATP-ligated form of DnaK (Hsp70) and stimulating the low-level ATPase activity necessary for molecular chaperone action. This is the only known function of DnaJ. The defining feature of DnaJ is the J-domain, approximatly 75 amino acids centered on the tripeptide histidine-proline-aspartate (HPD). All J-domains contain this HPD tripeptide, which is necessary for structure and biological function. The *Arabidopsis thaliana* genome contains a wide variety of J-domain proteins, most of which have not yet been characterized. atDjB48 is one of these proteins. Homologous genes have been identified in other species, but none has been characterized other than by sequencing. Total RNA was isolated from stems, roots, leaves, and flowers of *A. thaliana* to use as the template for reverse-transcriptase PCR (RT-PCR). The resulting DNA was most abundant with RNA from flowers. This was used as the template for a second round of PCR. The second PCR product was ligated with the pAT vector pCR-4-TOPO. This construct was used to transform *E. coli*. The vector was then isolated, and the insert was sequenced in both directions. This sequence perfectly matches the sequence previously annotated by the *A. thaliana* genome project. A vector for expression of the atDjB48 protein in *E. coli* is being constructed.

Adam Alter

Hometown: Jefferson City, Missouri

Major: Biology

University: University of Missouri-Columbia

Faculty Mentor: Dr. Helen Mullen, Molecular Microbiology & Immunology

Funded by Life Sciences Undergraduate Research Opportunity Program

The outcome of resolution versus fibrosis in granulomatous experimental autoimmune thyroiditis is associated with the cytokine profile

Adam Alter and Helen Mullen

The adoptive transfer model of granulomatous experimental autoimmune thyroiditis (G-EAT) studied by Dr. Mullen's laboratory has facilitated a detailed analysis of the pathophysiological mechanisms of autoimmunity. This study specifically set out to determine the roles of IFNγ and chemokines in determining the outcome of G-EAT. Following transfer of mouse thyroglobulin (MTg)-sensitized donor spleen cells activated with MTg and IL-12, recipient mice develop thyroid lesions at day 19-21 with granulomatous histopathology in both wild type IFNγ$^{+/+}$ (WT) and interferon-γ knockout (IFNγ$^{-/-}$) DBA/1 mice. These granulomatous inflammatory lesions evolved over time to two distinct outcomes, resolution or fibrosis. In WT mice, inflammation was persistent and thyroid fibrosis developed in 35-45 days after cell transfer. In IFNγ$^{-/-}$ mice, however, G-EAT lesions resolved by day 35-45. The gene and protein expression of chemokines was detected in G-EAT thyroids at various times by reverse transcriptase polymerase chain reaction (RT-PCR) and histoimmunostaining in order to assess the molecular mechanisms underlying the outcomes of either resolution or fibrosis. The results showed that IFNγ$^{-/-}$mice express undetectably low amounts of IFNγ, and expression of IP-10 (IFNγ inducible protein-10 kilodaltons), MIg (monokine induced by IFN-γ), CXCR3, MCP-1 and TGFβ are lower in IFNγ$^{-/-}$ mice than in WT mice. High levels of chemokine and chemokine receptors (e.g. IP-10, MIg, CXCR3) in thyroids of WT mice may act as dominant factor in directing inflammatory cells into thyroids, resulting in production of proinflammatory cytokines and the fibrotic molecules (TGFβ and MCP-1), contributing to development of thyroid fibrosis in G-EAT. Decreased expression of some chemokines in IFNγ$^{-/-}$ mice may result in less recruitment of effector cells to thyroids, contributing to the resolution of G-EAT. Insights gained from these studies may suggest novel therapeutic strategies for human autoimmune diseases.

Jonven J. Attia

Hometown: Brooklyn, New York

Major: Biology

University: Kingsborogh Community College

Faculty Mentor: Dr. Cathy Krull, Biological Sciences

Funded by National Science Foundation - REU (Life Sciences)

EphB2 may influence motor axon growth through the somites

Jonven Attia, Melissa Douglas, Rebecca McLennan, Sinead O'Connell, and Catherine Krull

During neural development, motor axons grow precisely to their final target regions to innervate muscle. We are interested in identifying the molecules that guide motor axons and in understanding how they work. Members of the Eph family of receptor tyrosine kinases and their ligands, the ephrins, are thought to play key roles in motor axon guidance. Recently, we discovered that the EphB2 receptor tyrosine kinase (RTK) was expressed by motor axons as they traveled through the somites. To test whether EphB2 RTK is required for motor axon guidance, we blocked EphB2 function using specific peptides that interfere with EphB2 binding to its ephrin ligands in trunk explants. Control explants were incubated in the absence of the EphB2 peptides. After growing trunk explaEphB2 may influence motor axon growth through the somites in vitro for 20-24 hours, the explants were fixed in 4% paraformaldehyde and stained with anti-neurofilament antibody to mark all axons, and anti-ephrin-B2 antibody, to label the posterior half of the somites. In control explants, motor axons entered the somites at their correct time and position; analyses are in progress to examine the effects of disrupting EphB2 signaling on motor axon growth. We also have begun to examine the expression of EphB2 in greater detail during the stages that motor axons project through the somites to target limb muscles. EphB2 is expressed on axons as they stall and sort at the base of the limb. Together, these results suggest that EphB2 RTK is necessary for motor axon growth during neural development.

Rebekah Augustine

Hometown: Kutztown, Pennsylvania

Major: Environmental Science

University: University of Delaware

Faculty Mentor: Dr. Paul Porneluzi, Dr. John Faaborg, and Rick Clawson, Biological Sciences

Funded by Missouri Ozark Forest Ecosystem Project

A survey of post-breeding season abundance of mature-forest breeding neotropical migrant birds in early-succession clearcuts

Rebekah Augustine and Christy Bowersox

It has been noted by researchers that birds who breed in the mature forest regularly move into early-succession clearcut habitats at the commencement of the breeding season. The goal of this study is to determine if mature forest birds make use solely of the edge habitat where the forest meets the clearcut, or if they penetrate the clearcut's interior. To date, the purpose and extent of this movement is not yet fully understood.

This study is part of the avian ecology portion of the Missouri Ozark Forest Ecosystem Project, sponsored by the Missouri Department of Conservation and the University of Missouri. The data were collected by mist netting for birds throughout the month of July in several research plots located in the Current River and Peck Ranch conservation areas of southeastern Missouri. Twelve mist nets were monitored each day by four birding crews along both the perimeters and the interiors of the clearcuts. The nets in the perimeter net lines were arranged approximately fifty meters apart along one side, while the interior nets were placed along a path down the center of each clearcut. Our clearcuts are small, ranging in width from about one hundred to three hundred meters.

We will use the data to determine habitat preferences among the immigrant mature-forest birds. We expect to find that there is a higher proportion of mature-forest birds residing in the edge habitats than in the interior of the clearcut early-succession areas. We will also observe the presence of hatching year birds. When the quantity of hatch year birds present is considered in tandem with the number of adults encountered, more inferences may be made about the post-breeding season habits of these birds.

Ashley C. Baker

Hometown: Nevada, Missouri

Major: Plant and Soil Sciences

University: Oklahoma State University

Faculty Mentor: Dr. Joe C. Polacco, Biochemistry

Funded by Plant Genomics Internship at MU

Overexpression of soybean ureases in *Escherichia coli*

Ashley C. Baker, Ariel Goldraij, and Joe C. Polacco

Urease catalyzes the conversion of urea to ammonia, which is an important N source in plants. Urease is a metalloenzyme and requires Ni for activation. Accessory factors are required for Ni insertion into urease. Activation of plant urease in *E. coli* would aid in understanding the mechanisms of urease activation.

Two urease isozymes are present in soybean: tissue ubiquitous and embryo-specific. The aim of this project was to overexpress each urease in *E. coli*. This will allow us to study plant urease activation in comparison with bacterial systems where urease activation is better understood.

The first step was to clone cDNA using PCR techniques to incorporate each soybean urease structural gene into *E. coli* expression vector pET-28a and transform it into DH5α. Kanamycin resistant colonies were used to recover plasmids. Specific primers were used to amplify the insert with PCR to ensure the insertìs presence in the vector. Colonies positive for the insert were chosen to prepare plasmid for transformation of HMS 174, which is competent for protein overexpression under control of a *lacP* promoter. Cultures were induced with 1 mM IPTG and the protein profile was analyzed by SDS-PAGE. A new band close to 90 kDa and only found in induced cultures confirmed that the ubiquitous urease was successfully overexpressed. The same procedure to express embryo specific urease is in progress.

LaWanda Barnes

Hometown: Nashville, Tennessee

Major: Psychology and Art

University: Fisk University

Faculty Mentor: Dr. Amanda Rose, Psychology

Funded by Louis Stokes Missouri Alliance for Minority Participation

Borderline personality disorder: Investigates gender, grade, and the number of friends, and the friendship qualities

LaWanda Barnes, Amanda Rose and Lance Swenson

The present research investigates whether children and adolescents who are high in borderline personality disorder (BPD) features have problematic friendship adjustment. Specifically, youth high in borderline features are expected to have fewer friends and friendships of lower quality than youth low in borderline features. Participants in third, fifth, seventh, and ninth grades (N = 1041) completed a personality assessment questionnaire assessing borderline personality features. In addition, friendship adjustment was assessed with two questionnaires, which determined the number of reciprocal friendships each participant had and assessed positive and negative qualities of their relationship with their best friend. The findings generally supported our hypotheses. Youth with high levels of BPD features tended to have fewer friends and more conflicts within their friendships than students with low features of BPD. Surprisingly, youth with high levels of BPD features also reported more positive qualities in their friendships. In addition, gender and grade differences in the levels of BDP features reported were examined.

William S. Beatty

Hometown: Maryville, Illinois

Major: Fisheries and Wildlife

University: University of Missouri-Columbia

Faculty Mentor: Dr. Paul Porneluzi, Dr. John Faaborg, and Rick Clawson, Biological Sciences

Funded by Missouri Ozark Forest Ecosystem Project

The presence of predatory mammals in a managed oak-hickory forest in Shannon County, Missouri

William S. Beatty and Matthew C. Miller

Our study was designed to investigate the effects of two different forest management treatments, no-harvest and even-aged management (partial clearcutting), on populations of predatory mammals. Our study occurred in conjunction with the Missouri Ozark Forest Ecosystem Project (MOFEP), a long term analysis of the impacts of forest management in an oak-hickory forest ecosystem. One site of each management technique was utilized in this study. At each of the sites, five randomly placed bait stations using partially open cans of tuna were set up and monitored by infrared and motion activated cameras. The presence of animals and frequency of their visitation to the stations were recorded from the images taken by the cameras, over two successive days at each location. From these data, we compared the abundances of mammalian predator species in the no-harvest and even-aged management areas.

Justan L. Blair

Hometown: Salem, Missouri

Major: Biology

University: Central Methodist College

Faculty Mentor: Dr. Paul Porneluzi, Dr. John Faaborg, and Rick Clawson, Biological Sciences

Funded by Missouri Ozark Forest Ecosystem Project

Effects of water temperature on aquatic turtle populations on the Current River

Justan L. Blair, Matthew E. Rice, and Gregory T. Wann

The Current River is home to approximately 10 species of aquatic turtles. This river is supplied with spring water throughout it's course. The temperature of the water coming from these springs is much colder than the temperature of the river itself. The mixing of the spring water with the river causes the temperatures downstream of these springs to be colder than those upstream. Our project looked at the effects of these temperature changes on aquatic turtle abundance. We would expect that due to these turtles being exothermic they would prefer the warmer water temperatures above the springs over the colder ones downstream from the springs. This would suggest that there would be more turtles found above the springs, than below. For our study, we used the areas upstream and downstream of Round Spring, Alley Spring, and Blue Spring. For each spring, the river 400 meters above and 400 meters below was used. During each run water temperature was taken and observed turtles were counted. Three trials were used for each spring in order to calculate the number of turtles above and below the springs.

Chris Blanner

Hometown: Kansas City, Missouri

Major: Psychology

University: University of Missouri-Columbia

Faculty Mentor: Dr. J. Thomas Cunningham, Dalton Cardiovascular Research Center

Funded by Life Sciences Undergraduate Research Opportunity Program

The effects of water depravation on Jun D-staining in the Supraoptic nucleus

Christopher F. Blanner, Karen A. N. Higgs, Jennifer Cornelius, and J. Thomas Cunningham

Understanding the basic mechanisms involved in body fluid homeostasis leads to understanding of the dysregualtion of systems that may contribute hypertension and congestive heart failure. One factor involved in body fluid homeostasis is plasma volume, and a decrease in plasma volume due to dehydration has been shown to significantly increase and decrease a variety of genes related to the AP-1 transactivator family. Jun D, a member of the AP-1 family, has been shown to be either activated or suppressed following water deprivation. The present study tests the results of previous studies by comparing different Jun D antibodies following water depravation. We are predicting a high level of staining (more Jun D positive cells) in controls and decreased activity with water deprivation. Adult male rats were water deprived for 24 or 48 hours. Controls were give ad lib access to water. All rats were anesthetized and perfused transcardially with 4% paraformaldehyde and free-floating sections (40 um) were stained for Jun D immunocytochemistry using three different antibodies (Santa Cruz). The number of Jun D-positive cells were counted in each SON and averaged for each rat. The three antibodies were then compared. We are in the process of comparing the individual antibodies. Our prediction is that the antibodies will show decreased Jun D staining due to dehydration. The results of this study will help show if the differences in the previous studies were due to the specificity of the Jun D antibodies used.

James P. Bosanquet

Hometown: Ballwin, Missouri

Major: Biochemistry

University: University of Missouri-Columbia

Faculty Mentor: Dr. Jeffrey Skimming and Dr. Vincent DeMarco, Child Health/Cardiology

Funded by Life Sciences Undergraduate Research Opportunity Program

Alpha-lipoic acid inhibits intrapulmonary nitric oxide formation in endotoxemic rats

James Bosanquet, Vinay Rawlani, Tammy Strawn, Vincent DeMarco, and Jeffrey Skimming

Introduction : The antioxidant, alpha-lipoic acid has been shown to protect tissues from oxidative stress and subsequent activation of the pro-inflammatory transcription factor, NF-kappaB, and its gene products. Endotoxin causes oxidative stress, activation of NF-kappaB and exaggerated production of nitric oxide. We hypothesize that alpha-lipoic acid inhibits intrapulmonary nitric oxide over expression in endotoxemic rats by inhibiting NF-kB activation. **Methods** : Rats were randomly assigned to 3 groups: 1) no special treatment, 2) LPS only, and 3) pretreatment with lipoic acid (100 mgs/kg at t = -4 and -1 hours) and then LPS. After the animals were anesthetized, endotoxin (LPS 0.01 mg/kg) or vehicle solutions were administered intravascularly and a sample of exhaled gas was taken every 15 minutes analyzing it for nitric oxide concentration. After 150 minutes of LPS administration , the animals were sacrificed and the lungs and heart were snap-frozen. Levels of NF-kappaB and iNOS were measured in lung homogenates. **Results** : We found that lipoic acid attenuated endotoxin-induced increases in exhaled nitric oxide, iNOS, and NF-kappaB. **Conclusions** : These findings suggest that alpha-lipoic acid protects against endotoxin-induced overexpression of intrapulmonary nitric oxide by inhibiting activation of NF-kappaB.

Christy Bowersox

Hometown: New Columbia, Pennsylvania

Major: Biology

University: Lycoming College

Faculty Mentor: Dr. Paul Porneluzi, Dr. John Faaborg, and Rick Clawson, Biological Sciences

Funded by Missouri Ozark Forest Ecosystem Project

A survey of post-breeding season abundance of mature-forest breeding neotropical migrant birds in early-succession clearcuts

Christy Bowersox and Rebekah Augustine

It has been noted by researchers that birds who breed in the mature forest regularly move into early-succession clearcut habitats at the commencement of the breeding season. The goal of this study is to determine if mature forest birds make use solely of the edge habitat where the forest meets the clearcut, or if they penetrate the clearcut's interior. To date, the purpose and extent of this movement is not yet fully understood.

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We will use the data to determine habitat preferences among the immigrant mature-forest birds. We expect to find that there is a higher proportion of mature-forest birds residing in the edge habitats than in the interior of the clearcut early-succession areas. We will also observe the presence of hatching year birds. When the quantity of hatch year birds present is considered in tandem with the number of adults encountered, more inferences may be made about the post-breeding season habits of these birds.

Britton Boyd

Hometown: Atlanta, Georgia

Major: Biochemistry

University: Mississippi State University

Faculty Mentor: Dr. Gary Stacey, Plant Microbiology & Pathology

Funded by Plant Genomics Internship at MU

Proposed role of oligopeptide transporters in heavy metal resistance in *Arabidopsis*

Britton Boyd, Minviluz Stacey, and Gary Stacey

Peptide transporters are divided into three familes: the ATP-binding cassette (ABC) family, the peptide transport (PTR) family (transporting di- and tripeptides), and the oligopeptide transport (OPT) family (transporting tetra- and pentapeptides). Completion of the Arabidopsis genome sequence revealed that this plant has ten-times more peptide transporters than any other sequenced organism, implying that peptide transporters play important and largely unknown roles in plant growth and development. Nine OPT family members were identified in Arabidopsis, but their function remains to be identified. To this end, it would be useful to identify the physiological substrates transported by these proteins. Among the small peptides known to move within plants are phytochelatins (g-glutamyl-cysteine containing peptides), which are thought to play a role in detoxification of heavy metals. In order to investigate whether OPT transporters could mediate the movement of phytochelatins, we tested various T-DNA mutants in various opt genes for their growth in the presence of heavy metals (i.e., Cd, Zn, and Cu). Root elongation was determined as a measure of growth. Preliminary results indicated that some of the mutants either showed enhanced sensitivity or enhanced resistance to the presence of these heavy metals. These experiments are currently being repeated to confirm these interesting results.

Dana M. Buder

Hometown: Eureka, Missouri

Major: Biology

University: University of Missouri-Columbia

Faculty Mentor: Dr. Gary Stacey, Plant Microbiology & Pathology

Funded by MU Monsanto Undergraduate Research Fellowship

Studies on chitin signaling using synthetic promoters

Dana Buder, Jinrong Wan, and Gary Stacey

Synthetic promoters, containing defined regulatory elements, when fused to a reporter gene allow for the analysis of gene expression in response to specific signals. Nine different synthetic plant promoters were used in this study and fused to β-glucuronidase (GUS). These promoter elements were chosen to respond to different plant pathogens and/or elicitors. Chitin, found in the cell walls of plant pathogenic fungi, is a well-known elicitor of plant defense pathways. Transgenic *Arabidopsis* plants containing each of the nine different synthetic promoter-GUS fusions were constructed by *Agrobacterium*-mediated transformation. Stable transformation was confirmed by DNA isolation from leaf disks and subsequent analysis by polymerase chain reaction using primers specific to the transgene. In this way, independent transgenic lines were identified for each of the nine different constructs. These plants were allowed to self-fertilize and seeds were harvested, sterilized and plated. Currently, we are challenging these plants with chitin to analyze which, if any, will show enhanced GUS expression after treatment. In this way, we hope to better define the signaling pathways that respond to this plant defense elicitor.

Denise Cafiero

Hometown: Bayonne, New Jersey

Major: Biology

University: The College of Saint Elizabeth

Faculty Mentor: Dr. Grace Y. Sun, Biochemistry

Funded by National Science Foundation - REU (Life Sciences)

Effects of plant polyphenols on cytokine induction of secretory phospholipase A_2 mRNA in primary rat astrocytes

Denise Cafiero, Michael Jensen, and Grace Y. Sun

Astrocytes are the major cell type in the brain. These cells play an important role for the brain by providing physical support to the neurons, releasing growth factors, and mediating the nutrient need of neurons. Astrocytes are immune active cells and they respond to pro-inflammatory cytokines, including tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and interferon-γ (IFN-γ), which cause induction of many genes including secretory phospholipase A_2 ($sPLA_2$), an inflammatory protein induced in immune active cells as a result of inflammation. In humans, increased $sPLA_2$ is linked to a number of diseases including sepsis, arthritis, and arteriosclerosis. In the brain, many forms of injury can induce $sPLA_2$ and secreted $sPLA_2$ can cause damage to neurons. Induction of $sPLA_2$ by cytokines typically involves the NF-κB signaling pathway. Many fruits and vegetables are rich in polyphenols, which possess anti-oxidative and anti-inflammatory properties. In this study, we investigated the effects of three polyphenols: (1) resveratrol, enriched in grape, (2) curcumin, a compound from the plant *Curcuma Longa Linn* , and (3) genistein, a phytoestrogen enriched in soy bean, on their ability to inhibit cytokine-induced secretory phospolipase A_2 ($sPLA_2$) mRNA expression in astrocytes.

Primary rat astrocytes were cultured in 60 mm dishes and treated with a mixture of cytokines (TNF-α, IL-1β, IFN-γ). Polyphenols (resveratrol, curcumin and genistein) at 10 and 50 μM concentrations were added to the dishes 30 min before cytokine treatment. Cells were treated with polyphenols and cytokines for 16 hr before extraction of RNA. RT-PCR was performed and the DNA product was separated by electrophoresis using 1.5% agarose gel in Trisacetate buffer (TAE) containing ethidium bromide. Cytokine induced $sPLA_2$ mRNA. Resveratrol and genistein at 50 μM and 10 μM did not inhibit cytokine induced $sPLA_2$ mRNA. Although curcumin at 10 μM did not inhibit $sPLA_2$ mRNA, near complete inhibition was observed at 50 μM. These results indicate that among the three polyphenols tested, curcumin exerted the strongest inhibitory effect on cytokine induction of $sPLA_2$.

Nicole Campbell

Hometown: St. Louis, Missouri

Major: Biology

University: University of Missouri-Columbia

Faculty Mentor: Dr. Shanna Swan, Family & Community Medicine

Funded by Summer Undergraduate Breast Cancer Research Program

The difference in age at menarche observed between African American and Caucasian athletes as it relates to breast cancer risk

Nicole Campbell, Shanna Swan, and Pamela Hinton

Evidence suggests that early menarche is a strong risk factor for the development of breast cancer in women. Women who experience menarche before the age of 14 are at a 30% increased risk for developing breast cancer compared to women who experience menarche at the age of 16 or later. Breast cancer is said to be a hormone dependent disease, thus, the longer the period of estrogen exposure to breast epithelium due to an early menarche, the greater are a woman's chances of developing breast cancer. Traditionally, African American girls tend to begin puberty earlier and thus experience menarche earlier than Caucasian girls. This trend leads some to believe that the difference in age at menarche contributes to earlier development of breast cancer and lower survival rates of African American women.

Evidence also suggests that girls who are athletic (i.e. those who participate heavily in sports, dance, and gymnastics) experience menarche at a later age than inactive girls do. This is due to the lower body mass index (BMI) for athletic girls and the critical BMI that must be attained in order to prepare adolescent girls for reproductive maturity. Girls who are anorexic, very athletic or who are professional ballet dancers who drop below the critical BMI frequently experience amenorrhea or very irregular menstrual cycles (oligomenorrhea).

Division I athletes completed a questionnaire anonymously, and the information was used to assess such variables as their current age, race, age at menarche, and number of sports years before and after menarche. The variable that we called 'sports years' was calculated by taking the product of the number of sports that individual participated in and the number of years they were involved with the sport(s) and then summing them.

The average age at menarche for African American participants (n=12) was 12.6 years of age and for all others (n=152) the average age was 13.4 years (p=0.10). This was not strongly related BMI. The average number of menstrual cycles per year was lower in African American girls (mean=10.2) than all other races (mean=11.4). The percent of African Americans that experienced oligomenorrhea was 30.7, which was higher than the percent of Caucasians and other races (18.4). We also found a significant relation between the number of sports years before menarche and age at menarche (p=.02) which allowed us to conclude that as the number of sports years increased, the age at menarche was delayed.

Yasmin Ali Garcia Carbonell

Hometown: Cd. Victoria, Tamaulipas, Mexico

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University: Instituto Tecnologico de Cd. Victoria

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Funded by Missouri Ozark Forest Ecosystem Project

A comparison of focal species average and relative frequency in mist nets, in 2002 to 2003, Cardareva Evenaged

Yasmin Ali Garcia Carbonell and Federico A. Enriquez

The main objective of our study was to compare the average and relative frequency of the focal species captured in mist nets in clear cuts from 2002 to 2003. Twelve mist nets of 2 meters height per 12 meters length were set up on the sites from 6:00A.M. to 11: 00 A.M., during fourteen days. Every two days, the nets were changed to a different net line, except for the last four days, which were in a row. The twelve focal species were: Acadian flycatcher (*Empidonax virescens*), Kentucky warbler (*Oporornis formosus*), Ovenbird (*Seiurus auricapillus*), Red-eyed vireo (*Vireo olivaceus*), Wood thrush (*Hylocichla mustelina*), Worm-eating warbler (*Helmitheros vermivorus*), Blue-winged warbler (*Vermivora pinus*), Hooded warbler (*Wilsonia citrina*), Indigo bunting (*Passerina cyanea*), Prairie warbler (*Dendroica discolor*), White-eyed vireo (*Vireo griseus*), and Yellow-breasted chat (*Icteria virens*). The average, as well as relative frequency, of these species was calculated, determined and compared between 2002 and 2003. Our study was conducted in the clear cuts of Cardareva Evenaged, which is a part of the Missouri Ozark Forest Ecosystem. MOFEP is a study about the effects of forestry on the ecosystem. This project is conducted in the Current River Conservation Area on a yearly basis, every summer.

Leilani Castleman

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Major: Biology

University: University of Missouri-Columbia

Faculty Mentor: Dr. Dennis Lubahn, Biochemistry/Child Health

Funded by Louis Stokes Missouri Alliance for Minority Participation

Searching for new ERRβ Isoforms in the mouse model

Leilani Castleman, Wei Zhou, Jenifer Bogener, and Dennis Lubahn

It is well-known that estrogen is a risk factor for breast cancer. Hence, understanding the relationship between estrogen and breast cancer is significant for physiological and pathological pathways. Traditionally it is thought that the effects of estrogen are carried through estrogen receptors (ER), which belong to nuclear receptor superfamily. Furthermore, there is a subfamily of estrogen-receptor related receptors (ERRα, β, γ) that share target genes, coregulatory proteins, ligands and sites of action with ERs. All ERRs are orphan nuclear receptors with no naturally occurring ligands defined yet, and they actively influence estrogen pathways. Studying ERRα, β, γ will reveal their novel functions in estrogen-induced pathways. During an attempt to clone human ERRβ, one known isoform along with two new isoforms (unpublished data) were confirmed. Two of the isoforms demonstrated human specificity, and revealed a unique distribution pattern, that may lead to their novel function. Despite the human specificity of the isoform, in silico studies showed that there might be a similar splicing event exist in mouse (unpublished data). If the mouse isoform is present, a comparative study with the mouse model can be performed to identify its biological roles. Present breast cancer treatment, using tamoxifen, illustrates that its active metabolite represses the constitutive transcriptional activity of ERRβ. Therefore, characterizing ERRβ gene, especially its isoforms, will provide possible breast cancer treatment targets and also lead to a clearer understanding of the alternative exon splicing event.

Erin Cazel

Hometown: Palo Alto, California

Major: Biological Sciences

University: Stanford University

Faculty Mentor: Dr. Thomas E. Phillips, Biological Sciences

Funded by Summer Undergraduate Breast Cancer Research Program

The effect of MIP-1α on cancer cell differentiation

Erin Cazel and Thomas E. Phillips

Cytokines are small peptides that are well recognized for their involvement in leukocyte trafficking and activation. They were originally thought to be released only by leukocytes as a means of directing and augmenting the immune response of other leukocytes, but it has now been shown that epithelial cells can both express and respond to cytokines. The HT29 parent cell line is derived from a human intestinal epithelium carcinoma and used often as an *in vitro* model system in studies of cytokine expression and effects. The HT29-18N2 (N2) clone differentiates uniquely to goblet cells -- secretory cells whose differentiation can be easily quantified by the amount of intracellular mucin they produce.

The Phillips lab has previously shown that a two-week exposure of the N2 cells to 1 μM cyclosporine resulted in a 94% increase in differentiation as assessed by their levels of intracellular mucin. Furthermore, exposure to cyclosporine increased secretion of interleukin-8 (IL-8), a cytokine that is a powerful neutrophil attractant. 24-hour treatment of cyclosporine increased IL-8 levels in N2 cells from 78.26 to 224.76 pg/ml -- an increase of 187% relative to the control. In addition, the Phillips lab has found that exposure to 1 μM retinoic acid almost completely blocks the expression of the goblet cell phenotype. Secretion of IL-8 under 24-hour exposure to retinoic acid decreased to 38.51 pg/ml, 49.2% of control levels. IL-8 secretion by these cells appears to be correlated with their level of differentiation. Dwinell et al. (1999) demonstrated that treatment of HT29 cultures with macrophage inflammatory protein-1 alpha (MIP-1α) increased levels of IL-8. It is therefore postulated that MIP-1α increases IL-8 secretion by increasing differentiation. To test this hypothesis, we exposed N2 cultures concentrations of 0, 1, 10 or 100 ng/ml MIP-1α for 14 days. The degree of differentiation as a function of MIP-1α concentration was quantified by measuring the area of intracellular mucin per cell length in a cross-section.

Maggie S. Christ

Hometown: Imperial, Missouri

Major: Biology

University: University of Missouri-Columbia

Faculty Mentor: Dr. Mark Kirk, Biological Sciences

Funded by Arts and Science Undergraduate Research Mentor Program

Selective migration of neuralized mouse embryonic stem cells towards tumor cells and conditioned media

Maggie S. Christ, Amanda K. Swenson, Peter Serfozo, Bernie L. Maria, and Mark D. Kirk

Brain tumors are the most common cause of mortality from cancer in childhood. The limitations of current treatments of malignant brain tumors motivate development of novel therapies. The overall goal is to develop stem cell therapies for treatment of malignant brain tumors. Neural stem cells migrate towards areas of damage and inflammation in the nervous system as well as malignant brain tumors. Our project was to determine whether embryonic stem (ES) cells migrate towards different tumor cells or media conditioned by tumor cell lines.

The project was carried out using undifferentiated mouse ES cells and mouse ES cells that were neuralized using retinoic acid. The various tumor cells used include human derived glioma lines, N1321 and U87, and chemically induced rat glioma line, C6. *In vitro* chemotaxis assays and fluorescence microscopy were used to determine the extent of migration by stem cells towards various types of tumor cells or conditioned medium. Assays were done using a 48-chamber chemotaxis apparatus from Neuro Probe. Porous membranes (8 micron pore diameter) were submerged and coated with ECL. The ES cells were harvested in DMEM (stem cell media) and placed on top of the membrane; tumor cells or media conditioned by a tumor cell line was placed in a chamber below the membrane. The controls consisted of ES cells placed above tumor buffer, DMEM over tumor cells, and in one control no cells were included in either chamber, with DMEM placed over astrocyte tumor buffer. Nuclei of cells that migrated through the porous membrane were labeled with Hoechst 33358, TIFF files were generated from fluorescent images, and the total number of nuclei were counted using NIH Image.

To date, we have obtained the following results. For undifferentiated ES cells, no differences were seen between migration patterns towards the various tumor cell lines, media conditioned by the tumor cell lines, or culture media alone. As the ES cells became more differentiated (i.e., neuralized), fewer cells migrated across the membrane under all conditions, suggesting a decrease in motility by differentiated neural progenitor cells. However, by day 8 of the ES cell induction protocol, significantly more differentiated ES cells migrated towards the tumor cells and media conditioned by the tumor cells, when compared to DMEM alone (Dunnet Test; $P < 0.01$ and $P < 0.05$, respectively). In future experiments, we will test the selective migratory abilities of other stem cell lines toward tumor cells and factors they produce. This project was funded by the Sears Trust Fund.

Alexis D. Cody

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Major: Biology and Psychology

University: University of Missouri-Columbia

Faculty Mentor: Dr. Dennis Lubahn, Biochemistry/Child Health

Funded by Louis Stokes Missouri Alliance for Minority Participation

Phytoestrogen regulation of phase II enzymes in prostate cancer cell lines

Alexis Cody, Dr. Nader Shenouda, Pete J. Ansell, and Dennis Lubahn

Prostate cancer is an important public health problem in the USA, accounting for more than 184,000 estimated new cases and 40,000 deaths in the year 2000 alone. Prostate cancer is a candidate disease for prevention study because of the great advancements in technology; therefore the capability to manipulate it within a laboratory setting is greater.

Phytoestrogens are estrogens found in many plants which are commonly used in traditional medicine. These compounds may be both agonists and antagonists of estrogen receptors in humans. These estrogenic receptors are stimulated according to their cellular environments. Oxidative stress is a chemical reaction within the environment that disrupts protein barriers or receptors causing a mutation in the DNA, thus developing cancer.

It is important to understand the regulation of enzymes that protect against oxidative stress. Phase II detoxification enzymes (glutathione S-transferase and quinine reductase) many of which are regulated by the anti-oxidant response element (ARE), are known to protect cells from oxidative stress. The phytoestrogen Genistein will be tested in three concentrations for the regulation of phase II enzymes in human prostate cancer cell lines. The different concentration levels of each phytoestrogen will give an indication of agonists and antagonists.

We hypothesize that all levels of concentration of Genistein will protect the cell from further oxidative stress; therefore inhibiting the growth of the cancer cells. With this information, we hope to test other phytoestrogens and further or change herbal and nutritional therapies in the prevention of this deadly disease.

Katie Connolly

Hometown: Kansas City, Missouri

Major: Biology

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Faculty Mentor: Dr. Anand Chandrasekhar, Biological Sciences

Funded by Life Sciences Undergraduate Research Opportunity Program

Morphology of facial motor neurons in the zebrafish hindbrain

Katie Connolly, Stephanie Bingham, and Anand Chandrasekhar

As the vertebrate nervous system develops, new neurons migrate from their birthplace to other locations in the nervous system. Our lab studies the mechanisms underlying the migration of branchiomotor neurons in the zebrafish embryo. These neurons are found in the hindbrain (brainstem) and control the movement of the jaws and gills of the fish. A subtype called the facial branchiomotor neurons (FBMNs) migrate from their birthplace, rhombomere 4 (r4), to their final destinations, r6 and r7.

The long-term goal of this project is to identify the neurons that connect to the FBMNs, and thus potentially regulate their activity. As a first step, I have examined the detailed morphology of the FBMNs cell bodies and dendrites by retrograde labeling with the lipophilic dye, DiI. At 48 hours post fertilization, the FBMN cell bodies are found very medially in r6 and r7. The axons extend anteriorly into r4 and exit laterally out of the hindbrain into the hyoid arch. The FBMN has a very prominent dendrite that extends 3-4 cell diameters away from the midline to the lateral margin of the hindbrain.

We are currently examining FBMN morphology in the *trilobite* mutant, where FBMN migration is blocked and the motor neurons remain at their birthplace (i.e. r4). Analysis of FBMN morphology in *trilobite* mutants will indicate whether the neuronal inputs to the FBMNs also undergo reorganization in response to the aberrant location of the FBMNs.

Kimberly Crouch

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Major: Biology

University: University of Missouri-Columbia

Faculty Mentor: Dr. James English, Plant Microbiology & Pathology

Funded by Plant Genomics Internship at MU

Functional genomics of cytokinin degradation in plants

Kimberly Crouch, James T. English, and Kristin D. Bilyeu

Cytokinin oxidase/dehydrogenases (CKX) are enzymes that degrade the phytohormone cytokinin in plants, and thus contribute to the control of cell division and growth. It is of practical interest to understand the roles of cytokinin metabolism and of CKX function during embryo development in cereal grains. The biochemistry of CKX enzymes has been studied extensively. However, there have been few investigations of the genetics of CKX action. The model plant *Arabidopsis thaliana* contains several CKX homologs that can be divided into two groups: those that are targeted for secretion from the cell, and those that are non-targeted and are retained in the cytoplasm. In both Arabidopsis and rice, for which complete genome information is available, there are multiple copies of targeted CKX enzymes, but only a single gene codes for the non-targeted enzyme. Of the seven CKX homologs present in *A. thaliana*, only one, AtCKX5, lacks a predicted N-terminal signal peptide and thus is not secreted from plant cells. The objective of this project was to characterize the function of individual targeted and non-targeted CKX genes in the model plant, *A. thaliana.* We have identified T-DNA insertion lines that carry mutations in several AtCKX genes, including AtCKX5. We are determining the point of T-DNA insertion in selected CKX genes, using DNA extraction methods and PCR. After selecting homozygous mutants we have investigated phenotypic differences between the various mutants and wild type *A. thaliana.* In future work, we will analyze heterozygous mutants for segregation of alleles to confirm that the phenotypic differences are indeed due to the mutations in the specific AtCKX gene.

Shorouk F. Dannoon

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Funded by Stevens' Fellowship Summer Research

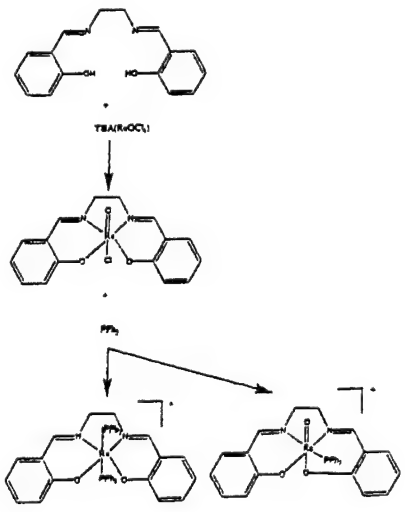
Synthesis of model Re(v) and Re(III) Schiff Base radiopharmaceutical complexes

Shorouk Dannoon, Hendrik Engelbrecht, and Silvia Jurisson

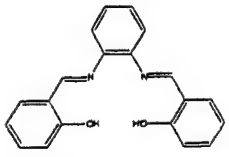
Our group research is focused on imaging and therapy radiopharmaceuticals that will target melanoma, breast cancer and prostate cancer. This is done by coordinating different metals such as Tc-99m, Re-188, Rh-105, Au-199, Pm-149, Ho-166 and Lu-177 to multidentate ligands. For these compounds to target the cancerous cells they must be kinetically inert under physiological conditions. For them to reach the wanted targets the complexes are conjugated to small biological molecules, such as peptides, that target particular cancer.

This research involved the synthesis of model complexes of these potential radiopharmaceuticals. Rhenium-188 and 186 are radioactive isotopes used for therapy, so the initial experiments were done with natural non-radioactive Re. N,Ní-ethuiene-1,2-bis(Salicylidinimine) (Sal2en) and N,Ní-phenyl-1,2-bis(Salicylidinimine) (Sal2Phen) are the two tetradentate Schiff base ligands that have been coordinated to Re to form the Re(V) complexes [ReOCl(Sal2en)] and [ReOCl(Sal2Phen)]. Phosphines were reacted with the Re(V) compounds to reduce the Re from oxidation state five to three which is more kinetically inert to form complexes of the type trans[Re(PR3)2(Sal2en/phen)]+ . The complexes were characterized by FT-IR, 1H NMR and 31P NMR spectroscopy.

Sal_2en Reaction:



Sal_2Phen ligand



Emily Dunn

Hometown: Bourbon, Missouri

Major: Biology

University: Truman State University

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Funded by Plant Genomics Internship at MU

Comparative study of Lepidopteron resistance in maize lines through protein analysis

E. Dunn, D. Davis, Q. Song, and G. Davis

Both the fall armyworm (FAW) and the southwestern corn borer (SWCB) have been known to cause significant yield loss in maize through the destruction of the whorl stage leaf. To date, several quantitative trait loci (QTL) corresponding to resistance from damage during the whorl-stage have been identified. One QTL corresponds to the *Glossy15* gene, which is a regulatory gene found to shorten the expression of the juvenile phase in maize leaves. Previously, protein patterns from parental lines that are resistant (Mp 705) or susceptible (Oh 28) to fall armyworm were analyzed through 2-deminsional gel electrophoresis. Comparisons were made focusing on the prescence/absence and relative intensity of protein spots. Differentially expressed proteins were identified through conventional methods (Soto, unpublished). For a more comprehensive study of the proteins involved in resistance to Lepidotera, the midgut of the FAW and SWCB exposed to either resistant and susceptible tissue was extracted and the protein analyzed. In addition preference tests were run to see if the Lepidopteron would choose one tissue source over another. The resulting data showed that *Glossy15* and *Glossy8* are genes that are directly correlated with Lepidopteron resistance. A wax reversal preference test was run to confirm that the wax layer of the mutants confers resistance. The goal of this comparative protein research is to identify proteins associated with Lepidopteron resistance in maize lines by narrowing the number of proteins found in the leaf extraction studies. These resistance proteins can then be integrated into maize lines in order to reduce the damage caused by these insects as well as reduce the cost of pesticides.

Christopher Durant

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Funded by MU Monsanto Undergraduate Research Fellowship

Selection of Sinorhizobium meliloti mutants defective in the biosynthesis of a novel symbiotic signal molecule, bradyoxetin

Christopher Durant, Monir Shababi, Brett Andersen, and Gary Stacey

Bacteria belonging to the genera *Rhizobium, Bradyrhizobium, Sinorhizobium* and *Mesorhizobium* develop a symbiotic relationship with their legume host. This process requires activation of nodulation genes (*nod*) expression in response to plant produced isoflavones. Previous work in our laboratory showed that induction of *nod* gene expression in *B.japonicum*was markedly reduced at high culture density. This repression is mediated through the action of a MerR-type protein, NolA, and a LysR-type protein, NodD2 (Fig.1). These two regulatory proteins are maximally induced at high population density due to accumulation of a unique signal molecule, designated bradyoxetin (i.e., 2-{4-[[4-(3-aminooxetan-2-yl)phenyl] (imino)methyl]phenyl}oxetan-3-ylamine). Bradyoxetin is chemically distinct from the acylhomoserine lactone quorum signals and is commonly produced by members of the alpha-proteobacteria (including *S. meliloti*). In order to elucidate the biosynthetic pathway leading to bradyoxetin production, *S. meliloti* mutants were selected that were defective in bradyoxetin activity. Mutants were isolated by Tn5 transposon mutagenesis. Two methods were imployed in the selection of muntants defective in bradyoxetin production. The first method utilized was the mating of Sinorhizobium (SM1021) with an E. Coli strain (SM10) which contains the Tn5 construct. Mutants that contain the insert of Tn5 are able to grow on media containing the antibiotic neomycin. These mutants are then mated with (pBGALAC4) which contains the nolA-lacZ plasmind. Mutants are selected on the inability to be induced by nolA-lacZ and will remain white in the presence of X-gal. Cloning and sequencing the DNA flanking the Tn5 insertion sites will allow the identification of the mutated gene by comparison to the full genome sequence of S. meliloti. Sequence comparison to the recently published complete sequence of *B. japonicum*should allow us to identify the homologous genes in this bacterium. In the second method, a cosmid DNA library, which contains the entire Bradyrhizobium genome including bradyoxetin genes was utilized and cosmid clones were obtained that contained the specific gene expression that was desired. The cosmid DNA library, pLAFR3, was prepared previously and was used to detect if the cosmid clones were able to induce the nolA-lacZ plasmid in *Bradyrhizobium japonicum* USDA 110-42. The availability of mutants blocked in *bradyoxetin* synthesis will be useful in further characterizing the role of this unique signal in the establishment of the symbiosis.

Mark Ebel

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Major: Biological Sciences

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Funded by Plant Genomics Internship at MU

Identification of srf-suppressor of rps4-RLD mutants that exhibit avrRps4-specific disease resistance

Mark Ebel, Soon Il Kwon, and Walter Gassmann

Plants have evolved multiple defense mechanisms on various levels to restrict growth of pathogens including viruses, bacteria, fungi, nematodes and protozoa. Gene-for-gene disease resistance is a highly specific plant defense mechanism. A plant exhibiting such resistance has a specific R gene governing its resistance response against only those pathogens expressing an analogous avirulence gene. The plantís recognition of the avirulence gene results in a defense response that ultimately results in the failure of the pathogen to destroy the plant. Work in our lab is focused on the Arabidopsis RPS4 gene specifying resistance to Pseudomonas syringae expressing the analogous avirulence gene avrRps4.

We have identified mutants that now display resistance that is specific to pathogens expressing avrRps4. To dissect the molecular mechanism of disease resistance, we are using a genetic approach to identify suppressor mutations that reactivate the RPS4-triggered plant defense response in RLD. The analysis of these mutants provides an opportunity to identify additional important genes in the Arabidopsis disease resistance signaling pathway.

Federico A. Enriquez

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Funded by Missouri Ozark Forest Ecosystem Project

A comparison of focal species average and relative frequency in mist nets, in 2002 to 2003, Cardareva Evenaged

Federico A. Enriquez and Yasmin Ali Garcia Carbonell

The main objective of our study was to compare the average and relative frequency of the focal species captured in mist nets in clear cuts from 2002 to 2003. Twelve mist nets of 2 meters height per 12 meters length were set up on the sites from 6:00A.M. to 11: 00 A.M., during fourteen days. Every two days, the nets were changed to a different net line, except for the last four days, which were in a row. The twelve focal species were: Acadian flycatcher (*Empidonax virescens*), Kentucky warbler (*Oporornis formosus*), Ovenbird (*Seiurus auricapillus*), Red-eyed vireo (*Vireo olivaceus*), Wood thrush (*Hylocichla mustelina*), Worm-eating warbler (*Helmitheros vermivorus*), Blue-winged warbler (*Vermivora pinus*), Hooded warbler (*Wilsonia citrina*), Indigo bunting (*Passerina cyanea*), Prairie warbler (*Dendroica discolor*), White-eyed vireo (*Vireo griseus*), and Yellow-breasted chat (*Icteria virens*). The average, as well as relative frequency, of these species was calculated, determined and compared between 2002 and 2003. Our study was conducted in the clear cuts of Cardareva Evenaged, which is a part of the Missouri Ozark Forest Ecosystem. MOFEP is a study about the effects of forestry on the ecosystem. This project is conducted in the Current River Conservation Area on a yearly basis, every summer.

Melanie D. Evans

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Faculty Mentor: Dr. William R. Folk, Biochemistry

Funded by Plant Genomics Internship at MU

Dissection of plant Pol III transcription machinery in *Arabidopsis thaliana*

Melanie D. Evans, Alexander Kenzior, and William R. Folk

The task of transcribing nuclear genes in eukaryotes is carried out by three RNA polymerases. RNA polymerase I (Pol I) transcribes genes encoding the large rRNAs. RNA Pol II transcribes genes encoding proteins involved in mRNA processing, and RNA Pol III transcribes genes encoding tRNA and 5S rRNA. All three enzymes are multisubunit proteins with their levels and activities subject to regulation during cell growth and development. Extensive studies of animal and yeast RNA polymerases have been accomplished while the plant RNA polymerases have been scarcely analyzed, especially Pol III. Pol III and its transcribed genes participate in many stages of the transmission of cellular genetic information, including transcription, translation, and RNA processing. It would be highly beneficial to define the proteins and DNA sequences responsible for plant tRNA gene expression and to determine how tRNA gene expression is regulated during plant growth and development. The long-term objective is to biochemically dissect the plant Pol III machinery and to reveal the mechanisms by which plant tRNA genes are transcribed and their expression regulated.

During my summer internship I mastered several essential molecular biology techniques and their underlying principles through research directed at the dissection of plant Pol III transcription machinery by means of three different approaches: 1) immunopurifying TFIIIC factor from transformed *A. thaliana* cell suspension cultures that had been previously developed with FLAG-tagged TFIIIC subunits TFIIIC63 and TFIIIC220. My contribution included growing these cell suspension cultures, preparation of media, and cell transfers. From these cells I prepared protein extracts, and utilized the method of immunopurification to isolate the protein of interest. To confirm the presence of the protein, I used the technique of SDS-polyacrylamide gel electrophoresis followed by Western Blot and visualization with luminescent substrate, 2) dissecting Pol III machinery by chromatin immunoprecipitation (ChIP) assay. The ChIP assay is a newly devised approach for the lab, and we are still in the beginning stages of optimization for our particular conditions. I ran preliminary experiments to determine the optimal length of sonication needed to shear the DNA to the desired fragment length of 500-1000 bp. At this point we have conducted the ChIP assay on AC40 and TFIIIC220 cell lines. To confirm the presence of immunoprecipitated genomic DNA, I employed the method of PCR followed by gel electrophoresis, and 3) capturing the Pol III machinery on tRNALys1 gene fragments immobilized on magnetic beads. PCR is used to amplify the tRNALys1 gene with biotin attached to the end of one primer. DNA fragments are then bound to streptavidin coated magnetic beads and mixed with WCE to capture Pol III machinery. I have encountered success in all three approaches, and feel confident in my newfound knowledge of these molecular biology techniques.

Jason R. Gentry

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Faculty Mentor: Dr. Charlotte L. Phillips, Biochemistry/Child Health

Funded by Life Sciences Undergraduate Research Opportunity Program

A comparative analysis of enzymatic activity of matrix matalloproteinases in heterotrimeric and homotrimeric collagen

Jason R. Gentry, Amanda C. Cully, and Charlotte L. Phillips

Osteogenesis Imperfecta (OI) is a connective tissue disorder characterized by mutations in the genes responsible for the synthesis of type I collagen. Type I collagen makes up part of the extracellular matrix (ECM) and is a heterotrimeric molecule, which is composed of two proα1(I) chains and one proα2(I) chain. The *oim* mouse model expresses homotrimeric type I collagen consisting of three α1 chains and has null expression of the proα2(I) collagen chain.

Studies of OI tend to focus on the musculoskeletal system, but this project is designed to examine kidney function in the *oim* mouse model. Normal production of type I collagen is regulated by matrix metalloproteinases (MMPs), which degrade ECM components including type I collagen. When the normal balance between ECM synthesis and degradation is disrupted, function of the glomerulus in the kidneys is impaired by increased type I collagen deposition resulting in mesangial cell death. Therefore, changes in MMP expression or activity result in altered ECM turnover and decline in normal filtration function of the glomerulus.

In this study, the aim is to compare heterotrimeric collagen and homotrimeric collagen degradation by specific MMPs. The specific MMPs found in the kidneys are MMP-1, 2, 3, 9, and 13. Eight percent zymography gels incorporating heterotrimeric collagen were produced so that analysis and examination of enzymatic activity for each specific MMP could be observed. A zymography gel made with homotrimeric collagen will be prepared to compare the two forms of collagen and how each form is degraded by the specific MMPs.

In order to prepare zymography gels, numerous amounts of isolated collagen is needed. Through experimentation, it was shown that more collagen could be extracted from wild type tails versus *oim* . Also, greater amounts of collagen are extracted from younger tails. Initially, rat type I collagen was used to demonstrate the migration and activity of MMP-1, 3, 8, and 9 to preserve the limited amounts of mouse collagen.

Future experimentation will involve a comparison between the enzymatic activity of MMPs produced in wild type and *oim* kidneys. This will show whether or not the MMPs involved in degradation of type I collagen are present in both kidneys.

Mike Gerau

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Funded by NSF Plant Genome Research Program Grant to G. Davis

Identification of QTL involved in *Zea mays* root response to drought conditions

M. Gerau, D. Davis, T. Musket, S. Pallardy, R. Sharp and G. Davis

Drought alone attributes to an average annual yield loss of 17% in maize (Zea mays). Improvement on annual yield is vital to securing the world food supply. In order to prepare farmers for the future, researchers have focused in on quantitative traits as key to solving the problem of an increasingly demanding world food market. While the quantitative traits of grain yield, ear number, and kernel number have received a great deal of attention the root system, however has for the most part been ignored. Root architecture plays a crucial in facilitating the uptake of water during a water deficit. Our goal was to identify the QTL for root architecture in maize and elucidate their role in drought response. Two experiments were performed. In the first experiment a subset of 94 mapping lines from the IBM population, was planted in five reps in a randomized complete block design in a peat base growth medium supplemented with a PAM water retainer under well-watered conditions for two weeks. After the two weeks, the plants were grown for ten days without watering. At the end of the ten day period primary root length, seminal root number, root branching, relative root water content, root mass, shoot mass, leaf number and the relative water content of the leaf tissue were measured. Genotypic data for 251 markers, evenly distributed throughout the genome, were used to construct a genetic map on Mapmaker Exp version 3.0 for Unix. QTL analysis was performed using QTL Cartographer Version 1.16. In the second experiment ten lines were used; the two parental lines B73 and MO17 and four lines with the largest average root mass as well as the four with the lowest root mass. The lines were grown under the method previously described and the same traits measured in addition to their water potential each day after the two week, well-watered period to correlate relative water content and water potential in the material. The QTL identified here can be used in marker-assisted-selection for improved drought tolerance.

Mark R. Goebel

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Major: Biotechnology/Molecular Biology

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Faculty Mentor: Dr. Joe C. Polacco, Biochemistry

Funded by Plant Genomics Internship at MU

In pursuit of allantoicase-negative *Arabidopsis* mutants

Mark R. Goebel, Christopher D. Todd, and Joe C. Polacco

Leguminous plants acquire nitrogen from the atmospheric N_2 by the symbiotic relationship with rhizobial bacteria in root nodules. The dominant transport compounds in many leguminous plants are the ureides, allantoin and allantoate. In nature, allantoate is catabolized by allantoicases. Allantoicase can be either allantoate amidohydrolase releasing ammonia (NH_3) or allantoate amidinohydrolase releasing urea. Either pathway would provide (NH_3), an important source of nitrogen, in plants.

An *Arabidopsis* mutant unable to degrade allantoate will be important for a better understanding of ureide metabolism. A search on SIGnal *Arabidopsis* database produced a line with a T-DNA insertion in a putative allantoate amidohydrolase structural gene. This gene showed homology to a bacterial (*Bacillus*) allantoate amidohydrolase. We examined plants of this line to recover homozygous segregants, lacking all expression of the disrupted gene. Homozygous plants were grown on several nitrogen sources including allantoin. Assuming that homozygous insertion lines have no gene activity, our preliminary results provide evidence that the knockout does not eliminate allantoate catabolism.

Merande M. Green

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Funded by Missouri Ozark Forest Ecosystem Project

Movement of worm-eating warblers (*Helmintheros vermivorus*) and black-and-white warblers (*Mniotilta varia*) from mature forest to clearcuts

Merande M. Green, Patrick Stanley, and Joseph Patson

During MOFEP 2003, in Reynolds and Shannon County, Missouri, we studied a potential trend in the movement of Worm-eating warblers (*Helmintheros vermivorus*) and Black-and-White Warblers (*Mniotilta varia*) from mature forests to clearcuts. Researchers have noted that these birds may be moving into the clearcuts with their fledged families. This is possibly because the clearcuts are good places for protection from predation and for foraging. Understanding how birds use the land during and after their breeding cycles is critical for the development of land management practices that can preserve them.

We examined spot map data from undisturbed and disturbed forest, as well as from clearcuts, to determine if birds were moving from mature forest to clearcuts. We counted the number of observances of the two species within three different two-week periods for the three separate types of landscapes. We then graphed the data for each landscape type to observe any trends. If forest interior birds are using these clearcuts, even-aged management may present some advantages to these species in addition to previously recognized benefits to early successional species.

Paul Hage

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Funded by Missouri Ozark Forest Ecosystem Project

Effect of forest management techniques on species richness and abundance of ground layer herbs

Paul Hage, Scott Loss, and Annie Silkowski

We compared species richness and abundance of ground layer herbaceous plants(excluding grasses) on sites with different forestry management techniques. The three sites studied were: even-aged (10-15% of timber harvested in several clearcuts that averaged 3-13 ha. in size), uneven-aged (10-15% of timber harvested in many small circular cuts 21-43 m. in diameter), and a control (no timber harvested). Ten random points on each site were picked where we used one-square-meter quadrats to collect data. Focus was placed on three species: Hogpeanut (*Amphicarpa bracteata*), Virginia Creeper (*Parthenocissus quinquefolia*), and Blackberry (*Rubus allegheniensis*). Species richness and abundances were determined and compared between the sites. These characteristics of plants may be a good predictor of overall species diversity and ecosystem health. This study was undertaken in the summer of 2003 in the Current River Conservation Area near Ellington, Missouri.

Kate E. Hart

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Major: Biochemistry

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Faculty Mentor: Dr. Judy Wall, Biochemistry

Funded by Plant Genomics Internship at MU

Comparison of the utilization of substrate by wild-type *Desulfovibrio desulfuricans* G20 and a cytochrome c_3 mutant CycA?

Kate E. Hart, Barbara J. Giles, and Judy D. Wall

The anaerobic sulfate-reducing bacterium, *Desulfovibrio desulfuricans* G20 is a naturally occurring soil microbe that may be useful for the bioremediation of toxic metals and radionuclides. This bacterium can change the redox state of a number of heavy metals, e.g. U, Te, and Cr, and thereby alter their solubility. The objective of this research was to explore the physiological role of the electron carrier protein, cytochrome c_3, which is a metal reductase in G20. The metabolism of a mutant of G20 lacking this cytochrome, CycA?, was compared to that of the wild type during batch culture. Consumption of the organic acid substrate lactate and production of the endproduct acetate was monitored by HPLC. The conversion of the terminal electron acceptor sulfate to its reduced form, sulfide, was also assayed during growth of the cultures. The CycA? strain grew to a lower cell density and generated less sulfide, although the lactate consumed was similar to the wild type. The copious amounts of hydrogen produced by the mutant may account for the differences between it and the wild type G20.

Rhea Hayden

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Faculty Mentor: Dr. Mark Flinn, Anthropology

Funded by MU Graduate School Summer Research Internship Program

The effects maternal stress has on health levels of children

Rhea Hayden and Mark Flinn

Studies with rodent and non-human primate models have found that pre- and post-natal stress, such as maternal separation, increase hypothalamic corticotropin-releasing factor (CRF) gene expression and hypothalamic-pituitary-adrenal (HPA) and behavioral responses (Meaney 2001; Suomi 1997). Studies with humans indicate that children of mothers with depression have similar patterns of abnormal neuroendocrine stress response (Essex et al 2002), but the developmental mechanisms are unknown. Dr. Mark Flinn has been conducting research for the past sixteen years on childhood stress and health in Bwa Mawego, a rural village on the island of Dominica. He has monitored the daily levels of cortisol, the primary hormone produced by the HPA system, by collecting more than 30,000 saliva samples from children in the village. The purpose of this research is to investigate mothersí stress level during pregnancy and how the stress levels of their children are subsequently affected. The project will include the examination of the mothersí medical records to document weight gain and illness during their last pregnancy. Participant observation and detailed questionnaires about family environments will be used to assess pregnancy stress levels. These questions will be broken down month-by-month to assess whether there are sensitive periods in the ontogeny of HPA stress response. The childrenísí cortisol data will be analyzed with the maternal histories to determine relations between maternal environment and child stress response. The results and conclusion of this project are pending because the data has not yet been collected.

Henrick N. Horita

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Faculty Mentor: Dr. Kathleen Newton, Biological Sciences

Funded by Plant Genomics Internship at MU

Determining the basis of CMS-C in maize mitochondria through sequence analysis

Henrick Horita, Louis Meyer, Melissa Rugen, and Kathleen Newton

Cytoplasmic male sterile (CMS) maize plants function similarly to normal (NB) maize plants except for an inability to produce functional pollen. CMS is useful for producing hybrid corn because it eliminates the laborious process of detasseling. Unfortunately, one strain of CMS maize, CMS-T, was found to have adverse effects, such as a susceptibility to a specific fungal toxin. Chimeric genes from the mitochondria cause CMS mutations in plants. These genes are made from a combination of gene fragments, orfs, and gene promoter sequences that have been linked together. We are studying the mitochondrial DNA of CMS-C maize, which has no known deleterious side effects, to find the gene responsible for its sterility. We used bioinformatics to analyze the genomic data. We first aligned the sequences of CMS-C and NB, which provided us with the possible chimeric genes in CMS-C. We found 89 possible chimeric genes, however, we narrowed the number of probable, functional chimeric genes down to nine. This process involved pairwise blasts, sequence alignments, and sequence arrangements. Future experiments will involve creating probes for these nine chimeric genes and hybridizing them to RNA gels of CMS-C mitochondria, and CMS-C with a nuclear gene that represses the male sterility, a restorer of fertility (Rf) gene. If a geneís RNA expression is changed in CMS- C, but not in CMS-C-Rf we can consider it to be a candidate gene for CMS-C.

Ivy Huntley

Hometown: New Orleans, Louisiana

Major: Mathematics

University: Dillard University

Faculty Mentor: Dr. Alex Iosevich, Mathematics

Funded by Louis Stokes Missouri Alliance for Minority Participation

Problem solving: Theory and practice

Ivy L. Huntley and Alex Iosevich

The main purpose of this project is to improve Ivy L. Huntley's problem solving techniques. We believe that solid understanding of the background material is necessary to accomplish this goal. Therefore, we combine theoretical derivations with hands on problem solving throughout the project.

Our main tools are pen and paper. In addition, we use several advanced mathematical texts focusing on number theory and elementary combinatorics. These books provide us with interesting theoretical concepts and serve as a source of exercises. A problem-solving book designed for national and international mathematics competitions is first on our agenda. We shall then move on to more advanced texts on continued fractions, if time allows.

As a result of this project, Ivy L. Huntley will gain deeper appreciation for mathematical rigor and a connection between theoretical and practical aspects of mathematics.

Jeffrey T. LaCroix

Hometown: Springfield, Illinois

Major: Biological Engineering

University: University of Missouri-Columbia

Faculty Mentor: Dr. Andrew McClellan, Biological Sciences

Funded by Life Sciences Undergraduate Research Opportunity Program

Using calcium imaging to monitor activity in reticulospinal neurons of the lamprey locomotor system

J.T. LaCroix and A.D. McClellan

The locomotor system in vertebrates consists of a locomotor command system in the brain that activates spinal central pattern generators (CPGs), which produce the basic pattern of activity that causes muscles to contract in a coordinated fashion (Grillner, 1981). The lamprey is a lower vertebrate with a simple nervous system that has many of the basic features of the CNS in more complex vertebrates, but with fewer neurons. In the lamprey, reticulospinal (RS) neurons in the brain are the main outputs of the command system that activate the spinal CPGs during swimming. In order to further understand the operation of the locomotor command system in the lamprey, it is important to determine the locations of neurons in the network, as well as the properties, connectivity, and patterns of activity for these neurons. A method of determining the electrical activity of these neurons involves the use of calcium indicator dyes. As neurons become active, there often is an influx of calcium, which can bind with an indicator dye, resulting in a change in fluorescence that can be imaged and analyzed as patterns of neuronal activity in the brain (McClellan et al., 1994). In this way, calcium indicator dyes and fluorescent imaging provide a method of observing the activity of large numbers of brain neurons. In the present study, Calcium Green-dextran amine was applied to the spinal cords of larval lamprey and was retrogradely transported to label RS neurons in the brain. After sufficient transport time, the lamprey brain and rostral spinal cord were removed and placed flat on a slide such that the RS neurons were visible on the dorsal surface. The slide was placed on a microscope equipped for fluorescence, and the caudal end of the spinal cord was briefly stimulated electrically, activating the Calcium Green labeled RS neurons. There was an increase in fluorescence of labeled RS neurons that was magnified by an intensifier and recorded by an S-VHS video camera and analyzed offline. The results show fluorescent increases in each of the nuclei of the brain containing RS neurons. For technical reasons, when using Calcium Green-dextran, it is not possible to record locomotor activity and correlate this with calcium imaging. However, this experiment provides groundwork for the next step, in which a lipid soluble calcium indicator dye, Calcium Green AM, will be applied to local areas of the brain in a semi-intact preparation to label small populations of neurons. This type of calcium imaging will provide a means to observe large groups of active brain neurons during actual swimming movements in these semi-intact preparations. The information obtained from these experiments will be used to better understand the operation of the locomotor command system not only in the lamprey, but in higher vertebrates as well.

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Funded by Arts and Science Undergraduate Research Mentor Program

Radiopharmaceutical research: Coordination of Re to tetradentate ligands

Stephanie R. Lane, Hendrik P. Engelbrecht, and Silvia S. Jurisson

Radiopharmaceuticals, drugs containing a radionuclide, are an effective method for both the diagnostic and therapeutic treatment of cancer. The radiopharmaceuticals under investigation consist of a radioisotope coordinated to a ligand, and are linked to a biological targeting molecule. Different biological molecules can be used that target breast cancer, prostate cancer or melanoma tumor cells. It is important for the radiometal to form a kinetically inert complex so that it will not irradiate other parts of the body. A radiopharmaceutical that will more effectively irradiate the tumor will provide better treatment and less overall discomfort for patients.

The focus of this study was to synthesize tetradentate ligands to effectively contain/bind the Rhenium (Re) metal. First, the exchange of oxygens for sulfurs in $acac_2en$ (figure 1) was investigated. The purpose was to increase the electron density on Re and possibly enhance reduction. When the resulting reactions resulted in low yields, a new approach was taken. Second, Re was reacted with sal_2pn starting ligands (figure 2) to form complexes of the type $[Re^{V}OCl(sal_2pn)]$. These Re complexes were then reacted with phosphines with the intent to produce a Re^{III} metal center, which is more stable than the previous Re^{V} metal center. Characterization methods used included IR, ^{1}H NMR, ^{31}P NMR and X-ray crystallography.

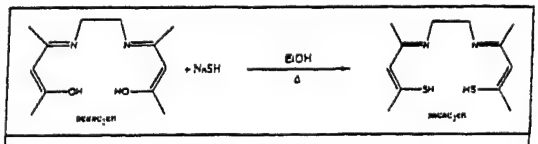

Fig. 1 Exchange of O for S in $acac_2en$.

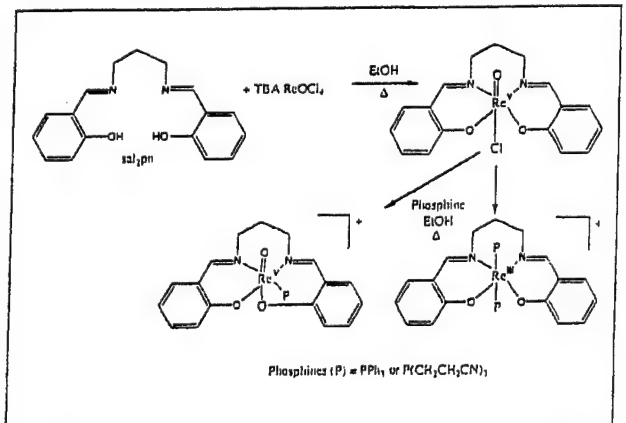

Fig. 2 Re reaction of sal_2pn, then reacted with phosphines.

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Funded by NIH Grant to G. Sun

Upregulation of secretory phospholipase A_2 in focal cerebral ischemia

Ming-H Lin, Qun Wang, and Grace Y. Sun

Secretory phospholipase A_2 ($sPLA_2$) is known to associate with inflammatory responses in a number of disease processes including stroke in the brain. In this study, the rat focal cerebral ischemia-reperfusion model is used to investigate whether ischemia enhances $sPLA_2$ and the cell type responsible for the increased expression of this protein. Male Long-Evans rats, weighing 250-300 g, were ligated at the trunk of the right middle cerebral artery with a 10-0 suture for 60 min. After ischemia, the suture was removed and blood flow restored. Three days after ischemia, rats were transcardially perfused and fixed. Coronal sections were cut at the dorsal hippocampal area. Brain sections were mounted on microscope slides to be used for staining. After deparaffinization in xylene, brain sections were hydrated in graded ethanol. Normal goat serum (NGS) and normal donkey serum (NDS) in PBS were used as a pre-blocking agent prior to applying the primary antibody overnight. Besides polyclonal antibody for $sPLA_2$, antibody for glial fibrillary acidic protein (GFAP) was used to identify astrocytes, isolectin-B4 for microglial cells, and DAPI for nucleus. Double immunohistostaining was performed with brain sections to localize $sPLA_2$ in astrocytes or microglial cells in the infarct and peri-infarct areas using an Olympous IX-70 regular light and BioRad Radiance 2000 laser scanning confocal microscope. A large infarction was found in the neocortex at 3 days after focal cerebral ischemia-reperfusion. Immunohistochemical study showed an increase $sPLA_2$ expression in the peri-infarct area and colocolized with GFAP-positive astrocytes. Although microglial cells were also increased, mainly in the infarct area, no obvious detection of $sPLA_2$ immunoreactivity could be observed. These results show that although both astrocytes and microglial cells respond to cerebral ischemia, upregulation of $sPLA_2$ is found only in reactive astrocytes.

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Effect of forest management techniques on species richness and abundance of ground layer herbs

Scott Loss, Paul Hage, and Annie Silkowski

We compared species richness and abundance of ground layer herbaceous plants (excluding grasses) on sites with different forestry management techniques. The three sites studied were: even-aged (10-15% of timber harvested in several clearcuts that averaged 3-13 ha. in size), uneven-aged (10-15% of timber harvested in many small circular cuts 21-43 m. in diameter), and a control (no timber harvested). Ten random points on each site were picked where we used one-square-meter quadrats to collect data. Focus was placed on three species: Hogpeanut (*Amphicarpa bracteata*), Virginia Creeper (*Parthenocissus quinquefolia*), and Blackberry (*Rubus allegheniensis*). Species richness and abundances were determined and compared between the sites. These characteristics of plants may be a good predictor of overall species diversity and ecosystem health. This study was undertaken in the summer of 2003 in the Current River Conservation Area near Ellington, Missouri.

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Does the hormone abscisic acid maintain or inhibit plant growth under water deficits? Analysis using NCED knock-out mutants of *Arabidopsis*

Rachel C.A. Maltman, Donald R. McCarty*,Bao Cai Tan*, Leina Joseph*, and Robert E. Sharp. *Horticultural Sciences Department, University of Florida-Gainseville, Gainseville, FL

The phytohormone abscisic acid (ABA) accumulates in plants under stress conditions, such as drought. The conventional view was that ABA accumulation functions to inhibit plant growth because well-watered plants slow their growth when ABA is applied. However, in studies of maize, the use of inhibitors of ABA synthesis and ABA-deficient mutants to decrease endogenous ABA levels showed that ABA functions in root growth maintenance under water deficits. Depending on the developmental stage of the plant, ABA was a cause of shoot growth inhibition in drought conditions. The first committed step in the biosynthesis of ABA is the oxidative cleavage of 9ί-*cis*-neoxanthin to xanthoxin, which is catalyzed by the enzyme 9-*cis*-epoxycaratenoid dioxygenase (NCED). To discover the function of the different members of the NCED gene family in root and shoot growth responses to water deficits, *Arabidopsis* knock-out mutants in the NCED genes were created by Donald McCarty at the University of Florida. Studying the NCED mutants will lead to a more complete understanding of the function of ABA in plant growth under water deficits.

All NCED mutants showed primary root growth promotion under water deficits compared to the wild type, which is contrary to what is known about ABA deficient maize seedlings. Not only was shoot growth inhibition by water deficits eliminated in the NCED mutants, but shoot growth was promoted by water deficits in NCED5 4250. These findings indicate that ABA accumulation is an important cause of inhibition of root and shoot growth in *Arabidopsis* seedlings under water deficit conditions. Measurements of root and shoot ABA content are being conducted to confirm these conclusions.

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Mechanisms by which glutamate causes neurite retraction of lamprey descending brain neurons in culture

G. Marsh, S. Ryan, L. Shotts, and A.D. McClellan

Locomotor behavior in the vertebrate nervous system is controlled by two major components: (1) spinal central pattern generators (CPGís) produce the basic pattern of locomotor activity; and (2) a locomotor command system in the brain activates the spinal CPGís to initiate locomotor behavior. Reticulospinal (RS) neurons are the output neural elements of the command system. After a severe spinal cord injury, the axons of RS neurons are severed resulting in paralysis below the lesion. In the lamprey, a lower vertebrate, following a spinal cord transection, descending brain neurons, including RS neurons, regenerate their axons, and there is recovery of locomotor behavior. These descending brain neurons can be grown in cell culture, and neurites from these neurons extend processes, perhaps by mechanisms similar to those during axonal regeneration.

In previous experiments, we showed that the neurotransmitter glutamate causes the neurites of lamprey RS neurons in culture to retract, presumably by increasing intracellular calcium. Glutamate might increase intracellular calcium by several mechanisms: activation of chemically-gated calcium channels; activation of intracellular signaling pathways that cause release of calcium from intracellular stores; or activation of voltage-gated calcium channels. In the present study, we investigated whether activation of voltage-gated calcium channels can cause neurite retraction.

A retrograde fluorescent tracer, DiI, was applied to spinal cords to pre-label RS neurons, and after 2-4 weeks, the brains were removed and triturated. Isolated RS neurons were plated in L15 media with serum (n = 23 neurons from 4 brains). Concentrations of extracellular potassium from 2-10 times normal were added to media. High potassium will continuously depolarize neurons and should open voltage-gated calcium channels. Time-lapse images were taken of the neurons to monitor growth or retraction of neurites.

Preliminary results suggest that elevated extracellular potassium does not cause significant neurite retraction. This result could be due to several reasons: voltage-gated calcium channels may have opened but then inactivated; potassium concentrations may have been too low to cause significant depolarization and calcium influx; or voltage-gated calcium channels may not contribute significantly to neurite retraction. In the future, high potassium solutions will be to repetitively pressure eject onto neurites of RS neurons to remove inactivation and allow voltage-gated calcium channels to reopen. The data from these experiments will lead to a better understanding of the mechanisms that regulate neural regeneration in the lamprey as well as higher vertebrates.

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Mapping inputs to reticulospinal neurons in the locomotor command system of the lamprey

A. McCroskey and A. McClellan

Brain locomotor command systems integrate information and send descending axons to central pattern generators (CPGs) in the spinal cord to initiate muscle contractions and locomotor behavior. Reticulospinal (RS) neurons are the main output elements of the brain command system that activate the spinal CPGs. To understand the operation of the locomotor command system, it is important to determine where the relevant neurons are located, as well as how they are interconnected and their properties.

The lamprey, a lower vertebrate, has a number of powerful advantages for studying the operation and organization of locomotor command systems that initiate swimming behavior. In the present study, anatomical techniques were used to identify neurons in the lamprey brain that have inputs to RS neurons. Larval sea lamprey (P. marinus) were anesthetized, and the brains and rostral spinal cords were removed. The tissue was pinned dorsal-side up on a small strip of Sylgard and transferred to an experimental chamber. Texas Red dextran amine (TRDA), a fluorescent tracer, was applied in the vicinity of RS neurons in the following reticular nuclei: anterior (ARRN; n = 7), middle (MRRN; n = 11), and the posterior (PRRN; n = 7) rhombencephalic reticular nuclei. The rationale was to allow the tracer to be taken up by axons that project to RS neurons and retrogradely transported to the cell bodies of input neurons. Specifically, crystals of the tracer were applied to single reticular nuclei. After retrograde transport of the TRDA, the brains were histologically processed and viewed using fluorescent microscopy.

Tracer application to reticular nuclei resulted in labeled neurons in two main areas of the brain: (a) lateral rhombencephalon contralateral to the tracer application site; and (b) mesencephalon and perhaps the diencephalon, mostly ipsilateral to the tracer application site. Neurons in the lateral rhombencephalon probably represent second order trigeminal sensory neurons that are involved in eliciting flexure responses. Neurons in the mesencephalon and diencephalon probably represent higher order locomotor control centers in the command system. A determination of the inputs to RS neurons will contribute to our understanding of how the locomotor command system operates in the lamprey as well as higher vertebrates.

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Testing substrate selection as an indicator of habitat preference in newly metamorphosed Southern Leopard Frogs (*Rana sphenocephala*)

Andrea L. Miller, Stacy M. James, and Raymond D. Semlitsch

Amphibians spend most of their lives on land, yet little work has been done on their terrestrial habitat preferences. If we are to help conserve declining amphibian populations, we must better understand their habitat requirements in all stages of life. Our experiment tested substrate selection by Southern Leopard Frog (*Rana sphenocephala*) metamorphs for two major habitats, field and forest, as well as their preference for wet or dry soil. During each trial, eight frogs were put into individual aquaria divided into two halves. The following three experiments were conducted simultaneously: 1) forest soil plus litter/field soil plus litter; 2) forest soil plus crushed litter/field soil plus crushed litter; and, 3) wet forest soil plus litter/dry forest soil plus litter. Frogs were left undisturbed overnight and their position on a particular side of the aquarium was recorded the next morning. Thirty out of 33 chose the forested side in the intact litter experiment. A lower number, 21 out of 33 frogs, chose the forested side in the crushed litter experiment. Twenty-one of 22 frogs chose the wet side of the hydration experiment, which indicates that cage-stress did not impede the frogs from making a choice. Therefore, the high number of forest choices can be interpreted as a preference for forested habitats. Furthermore, the stronger selection for forest in the intact litter experiment may indicate the importance of structure within a habitat rather than just odor. Overall, these results show that conservation and management practices concerning the Southern Leopard Frog should not only include their breeding ponds and other wetlands, but also the surrounding forested areas.

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Funded by Missouri Ozark Forest Ecosystem Project

The presence of predatory mammals in a managed oak-hickory forest in Shannon County, Missouri

William S. Beatty and Matthew C. Miller

Our study was designed to investigate the effects of two different forest management treatments, no-harvest and even-aged management (partial clearcutting), on populations of predatory mammals. Our study occurred in conjunction with the Missouri Forest Ecosystem Project (MOFEP), a long term analysis of the impacts of forest management in an oak-hickory forest ecosystem. One site of each management technique was utilized in this study. At each of the sites, five randomly placed bait stations using partially open cans of tuna were set up and monitored by infrared and motion activated cameras. The presence of animals and frequency of their visitation to the stations were recorded from the images taken by the cameras, over two successive days at each location. From these data, we compared the abundances of mammalian predator species in the no-harvest and even-aged management areas.

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Funded by Summer Undergraduate Breast Cancer Research Program

Characterization of Glc7 suppressors

Mary B. Millwood and John F. Cannon

Glc7 is a phosphatase that is known to be crucial to the cell cycle in budding yeast. Glc7 over expression kills the cell, however, suppressors have been found that will allow the cell to tolerate Glc7 over expression. *FPR3*, a dominant suppressor for Glc7 over expression, is found to suppress *tom1*, as well. Tom1 is responsible for transferring ubiquitin within the cell. The suppression of *tom1* by *FPR3*, led us to want to know whether or not other suppressors of Glc7 will work on *tom1*, as well. Cdc48 is found to have the same effects to the cell as Glc7 over expression, when it is mutated to *cdc48-S565G*. Cdc48 is, also, very important to the cellís cycle. The similar behavior of the two proteins made us want to know if the suppressors of Glc7 would suppress *cdc48-S565G*. Sds22 is known to bind to Glc7, in order to transport the phosphatase from the cytoplasm to the nucleus. Glc7 can, also be dominantly suppressed by the nonsense mutation called *SDS22-S56am*. Therefore, we wanted to know how Sds22 bound to Glc7, and the mechanism of how it suppresses. These experiments will allow us to explore the regulation of Glc7 and the cell cycles processes it regulates. These experiments are beneficial to the fight against all cancer, because we will better understand the process in which the cell divides, because by understanding how Glc7 operates we can have a better understanding of the cell cycle.

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Funded by Life Sciences Undergraduate Research Opportunity Program

The effects of angiotensin receptor antagonists in the rat rostral ventrolateral medulla

Andrew A. Mitchell and Cheryl M. Heesch

Under normal conditions and in response various perturbations, mean arterial pressure and heart rate are regulated by sympathetic activity. Preganglionic sympathetic neurons in the intermediolateral column of the spinal cord receive direct projections from areas of the brainstem and hypothalamus. One area of the brainstem in particular, the rostral ventrolateral medulla (RVLM), is responsible for the majority of sympathetic tone under basal conditions. The RVLM receives various excitatory and inhibitory inputs that contribute to sympathetic outflow, however, the excitatory inputs into the RVLM are less well understood. Angiotensin is thought to play a role in excitation of the RVLM, in part because the peptide angiotensin receptor antagonist, sarthran, produces profound sympathetic inhibition when microinjected into the RVLM. However, it has been demonstrated that this inhibition is not dependent on traditional angiotensin receptors (AT1 or AT 1-7). As others have proposed, this suggests that either a non-traditional angiotensin peptide receptor is involved, or that possibly an inhibitory glycine molecule is cleaved from sarthran following injection.

In our study, we sought to learn more about this mechanism by a microinjection regimen in rats in which we first injected sarthran into the RVLM, followed by strychnine, a glycine receptor antagonist. Following injection, changes were recorded in mean arterial pressure, heart rate, and renal sympathetic nerve activity. A reversal of the response to sarthran would indicate a role for glycine in sarthran induced sympathetic inhibition. To verify that the results were not due to blockade of endogenous glycine by strychnine, in a second injection regimen, strychnine was injected first, followed by sarthran. As previous studies indicated, strychnine alone should have little effect in the RVLM. If the effect of sarthran is attenuated in the presence of strychnine, this would strongly suggest that glycine was being cleaved off the sarthran molecule, and contributing to the observed response of sarthran in the RVLM. Further work in the Fall will strengthen the statistical significance of our findings.

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Funded by Life Sciences Undergraduate Research Opportunity Program

Female preference functions and mate choice based on call combination structure

Darah Oxford and Carl Gerhardt

The most common frog vocalization heard in H. *chrysoscelis* and H. *versicolor* is the advertisement call which functions to defend resources, to repel competitors, and to attract mates. Females choose males almost exclusively on the basis of the attractiveness of their signals which depend on one or a combination of factors including pulse rate, pulse shape and frequency. To understand the evolution of communication systems one must study signals and preference mechanisms. Sensory systems that have already evolved in a species make some signals easier to detect by receivers than others. Mutant signals similar to or having some same qualities as preexisting biases are more likely to cause evolutionary change in the communication system of the species when compared to signals that are not readily apparent by other individuals.

By using synthetic calls and digitized natural calls, parts of the calls (advertisement or aggressive) of other species in the gray tree frog group can be appended to advertisement calls of the species that is being tested to see if this might make the signal more attractive. The "pre-existing bias" can show the influence of considering phylogeny to explain the changes in signals and preference mechanisms by suggesting that some features of communication systems arose independently or by convergence. Intense selection on communication systems can lead to rapid divergence and speciation.

If hidden preferences are general, then the most widely accepted model for the evolution of communication systems - that of tight co-evolution of signals and preferences - will be called into question. Rather than gradual, step-by-step evolution - expected because large (mutational) changes in signals are more likely to make them ineffective rather than more attractive - pre-existing biases may allow rapid "innovations" and evolution in communication systems.

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Comparison of acoustic criteria for call recognition between *H. versicolor*and *H. chrysoscelis*

Jon Oxford and H.C. Gerhardt

The Gray Tree Frog complex is an ideal model system for studying how sender-receiver communication systems change over time. Acoustic communication in frogs is a major determinant of reproductive fitness. Males produce unlearned advertisement calls to attract gravid females, which have a set of acoustic criteria for choosing among the signals of different males in a chorus.

Both signal and receiver are under mutual selection, in that the signals are selected for on the basis of female criteria, while the female's criteria are propagated by reproduction. One consequence of this relationship could be that signals tightly co-evolve. However, in studies of closely related species, differences in female acoustic criteria suggest that this view of evolution and speciation is not universally true.

A key to understanding this issue lies with further quantification of the preferences of *H.chrysoscelis*.

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Funded by Louis Stokes Missouri Alliance for Minority Participation

Construction and evaluation of a novel biosensor for enzyme recognition

Maricruz Pajares, Susan Lever, Sheila Grant, Fabio Gallazzi, and Darcy Lichlyter

A new biosensor is being developed in order to detect medically important enzymes. Two specifically designed peptides substrates have been synthesized that contain an enzyme specific sequence. The peptide sequences will contain Fluorescein, a fluorescent dye that will work as an indicator to determine if cleavage, breakage, has occurred. A process called solid-phase synthesis is used to prepare the peptide. This is where the FMOC (Fluorenolmethoxycarbonyl) strategy, along with other protecting groups will be used in order to prevent the side chains of the amino acids from interacting. After the last FMOC has been removed, Fluorescein is added to the peptide sequence. Then a final chemical reaction will in one step remove the peptide from the resin as well as the other protecting groups. At that point thrombin is added, an enzyme that reacts at the specific cleavage point, and a change in fluorescent will be recorded. The first peptide, positive control, contains an arginine group, which will allow the enzyme to react and cleave the peptide. While the second group without arginine, negative control, will not react or cleave when the enzyme is added. The cleavage will be measured as a function of time. This process is very important because it is the first step in making an in-vivo biosensor to monitor Thrombin, a blood-clotting enzyme.

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Movement of worm-eating warblers (*Helmintheros vermivorus*) and black-and-white warblers (*Mniotilta varia*) from mature forest to clearcuts

Joseph Patson, Patrick Stanley, and Merande Green

During MOFEP 2003, in Reynolds and Shannon County, Missouri, we studied a potential trend in the movement of Worm-eating warblers (*Helmintheros vermivorus*) and Black-and-White Warblers (*Mniotilta varia*) from mature forests to clearcuts. Researchers have noted that these birds may be moving into the clearcuts with their fledged families. This is possibly because the clearcuts are good places for protection from predation and for foraging. Understanding how birds use the land during and after their breeding cycles is critical for the development of land management practices that can preserve them.

We examined spot map data from undisturbed and disturbed forest, as well as from clearcuts to determine if birds were moving from mature forest to clearcuts. We counted the number of observances of the two species within three different two-week periods for the three separate types of landscapes. We then graphed the data for each landscape type to observe any trends. If forest interior birds are using these clearcuts, even-aged management may present some advantages to these species in addition to previously recognized benefits to early successional species.

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Funded by Life Sciences Undergraduate Research Opportunity Program

Development of a potential imaging and radiotherapeutic agent for breast cancer: ^{111}In-DOTA-GA-KCCYSL

Holly Powell, Donna Whitener, Yubin Maio, Fabio Gallazzi, and Thomas Quinn

Approximately 182,000 women are diagnosed with breast cancer each year. There is a need to discover imaging agents that are more effective in early detection of breast cancer. A novel peptide sequence was identified from bacteriophage display library that binds to ErbB-2 receptors, which are often overexpressed on breast cancer cells. It is postulated that one could improve the selectivity of a diagnostic agent by covalently linking the agent to the peptide KCCYSL. This will allow the peptide to carry cancer-imaging and radiotherapeutic agents directly to the cells *in-vivo*.

In this study, we established the optimal radiolabelling conditions for synthesizing ^{111}In-DOTA-GA-KCCYSL and examined its stability *in-vitro*. The optimal radiolabelling conditions were found by varying the pH and concentration of the buffer, the incubation temperature, the incubation time, and the amount of peptide in the reaction. The stability of the complex was determined by adding it to various solutions that are similar to physiological conditions within the human body.

Our results demonstrated that the best complexation yield was obtained by heating the mixture of $^{111}InCl_3$ in 0.05M HCl with 0.5M NH_4OAc buffer of pH 5.4, and 10μg of peptide for 45 minutes at 75°C. We also determined that the complex was stable in physiological conditions. In conclusion, we now know that the complex is stable enough to perform cell-binding studies in the future.

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Funded by Summer Undergraduate Breast Cancer Research Program

sAPPα is released following activation of the $P2X_7$ receptor in 1321N1 astrocytoma cells

LaTasha A. Rabsatt, Jean M. Camden, and Gary Weisman

Alzheimerís Disease (AD) currently affects four million Americans and it is estimated that this number will increase to 15 million over the next decade. AD is characterized by amyloid plaques that aggregate and eventually destroy large areas of the brain leading to death. The B-amyloid precursor protein (APP) is an integral membrane protein that is present in the plasma membrane of both neurons and glial cells in the brain. This precursor protein has been linked to the formation of B-amyloid plaques.

The $P2X_7$ receptor, a ligand-gated ion channel activated specifically by extracellular ATP, is known to be present in brain neurons and glial cells, but little is known about the role of these receptors. Preliminary studies showed that $P2X_7$ receptor activation increases the release of sAPPα and we have attempted to determine the signal transduction pathway involved.

The recombinant rat $P2X_7$ receptor or control vector (pLXSN) was expressed in human 1321N1 astrocytoma cells that lack endogenous P2 receptors to determine if activation of the $P2X_7$ receptor subtype is linked to APP proteolytic cleavage. Cell transfectants were stimulated for two hours with BzATP, a highly potent, synthetic $P2X_7$ receptor agonist, and with various inhibitors of signal transduction pathways to assay for effects on sAPPα release into the medium. sAPPα release was detected by Western analysis using the 6E10 antibody (1:1000 dilution; Senetek, Maryland Heights, MO). Phorbol myristate acetate (PMA), an activator of PKC, was used as a positive control for activation of the PLC pathway. Cells expressing pLXSN were used as a negative control.

Results showed that BzATP caused the dose-dependent release of sAPPα into the medium of 1321N1 cells expressing the $P2X_7$ receptor, as compared to negative control cells. U0126, an inhibitor of MEK, the kinase that activates extracellular signal-regulated kinase (ERK), a mitogen-activated protein kinase (MAPK), partially blocked sAPPα release induced by BzATP indicating that ERK is part of the sAPPαrelease pathway. PMA stimulated sAPPα release in cells expressing $P2X_7$ receptors or pLXSN (negative control) indicating that activation of PKC is sufficient to stimulate α-secretase.

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Funded by Louis Stokes Missouri Alliance for Minority Participation

Creating a vector to knockout exons 7,8, and 9 of interleukin 13 receptor alpha 1 gene (IL-13Rα1)

Jeremy Raincrow and Andrei Alexenko

Neonatal tolerance occurs in neonatal mice and keeps them from mounting a regulatory immune response. IL-13 is believed to be a cytokine capable of inducing neonatal tolerance. We are establishing a lack of IL-13 effectiveness by creating a mouse void of the IL-13Rα1 subunit which is responsible for intracellular triggering of signal transduction. An IL-13Rα1 deficient mouse strain will be produced by creating a vector to knockout a critical fragment of the gene. This vector is created by first selecting a gene or critical fragment of the gene to be knocked out and selecting a length of DNA from both 5í and 3í ends of that gene to clone. The critical fragment selected for the IL-13Rα1 gene was exons 7, 8 and 9 and the flanking regions were 3.4kb 5í intron and 3.7kb 3í intron. The 3.7kb 3í intron has already been introduced into the intermediate vector known as pBlueScript Neo/TK which is a modification of the commercially available pBS KS vector. The 3.4kb 5í intron could not be run through PCR so it was broken into two fragments. The two fragments obtained were a 2.7kb fragment and a 1.35 kb fragment. The 1.35kb fragment has already been amplified. The 2.7kb fragment is now being amplified. There have been several problems amplifying this fragment. The 2.7kb fragment would not run through PCR. It has been introduced into a vector and amplified by bacterial transformation. We have not been able to re-isolate the 2.7kb fragment in its pure form from the bacterial cells.

Khandicia N. Randolph

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Faculty Mentor: Dr. Rebecca Martinez, Psychology

Funded by Louis Stokes Missouri Alliance for Minority Participation

Images of African American film and television actors in entertainment magazines: 1950-2002

Khandicia N. Randolph and Rebecca Martinez

Through various other studies that incorporate different magazines and subject matter it has been illustrated that there is an inequity in the imaging of African Americans in magazines. This inequity is derived from various factors that can be paralleled to the segregationistís practices in the United States and institutionalized racism. Such images are circumscribed from historical context relating back to the late Theodore Roosevelt and continued through infer orating depictions of African Americans in the early years of television. Research communicates to us that the largest difference can be found by viewing magazines whose readership varies by race and ethnicity. In this study I have sought out to prove that the images of these actors will be more positive and abundant in content in magazines whose readership is predominantly African American. All samples used in the qualitative research are deliberate. Each magazine was then viewed individually. The results that have been found are that there is a significant difference in the amount of images and articles that are found incorporating African Americans in predominately African American magazines than in predominantly Caucasian magazines or those that are centralized and that these images tend to focus on more positive and ant-stereotypical portrays of the race. Included in the analysis are statistics of the readership and demographics of the magazines. Conducting this research has been enlightening and rewarding. I have been able to develop concepts and ideas that can help society to understand the effects that both negative and positive images have on people and the human perspective.

Vinay Rawlani

Hometown: Columbia, Missouri

Major: Biochemistry

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Faculty Mentor: Dr. Vincent DeMarco and Dr. Jeffrey Skimming, Child Health/Cardiology

Funded by Life Sciences Undergraduate Research Opportunity Program

Hyperthermia inhibits intrapulmonary inflammation and preserves lung functionality in lipopolysacchride treated rats

Vinay Rawlani, James Bosanquet, Tammy Strawn, Vincent DeMarco, and Jeffrey Skimming

Introduction: Stress pre-conditioning has been shown to protect tissues from oxidative stress, activation of the transcription factor, NF-kappaB, and subsequent expression of pro-inflammatory gene products. Endotoxin causes oxidative stress, exaggerated production of nitric oxide and pro-inflammatory cytokines that can impair tissue function. We hypothesize that heat-shock preconditioning attenuates intrapulmonary nitric oxide over-expression and preserves lung function in endotoxemic rats. Methods: Rats were randomly assigned to 3 groups: 1) normothermia/non-LPS, 2) normothermia/LPS, and 3) hyperthermia preconditioned/LPS. After the animals were anesthetized, endotoxin (LPS 0.01 mg/kg) or vehicle solutions were administered intravascularly. Mean arterial pressure was recorded and samples of exhaled gas were analyzed for nitric oxide concentration every 15 minutes. Arterial blood oxygen tension and pH were measured at the time of endotoxin treatment and 150 minutes afterwards. Results: We found that hyperthermic preconditioning attenuated over-expression of exhaled nitric oxide, inhibited decreases in mean arterial pressure, and preserved lung functionality as shown by arterial pO2 and pH during endotoxemia. Conclusions: These findings suggest that heat-shock preconditioning protects against the over-expression of nitric oxide and functional-impairment of the lungs in endotoxemic rats.

Matthew E. Rice

Hometown: Slippery Rock, Pennsylvania

Major: Biology

University: Slippery Rock University

Faculty Mentor: Dr. Paul Porneluzi, Dr. John Faaborg, and Rick Clawson, Biological Sciences

Funded by Missouri Ozark Forest Ecosystem Project

Effects of water temperature on aquatic turtle populations on the Current River

Matthew E. Rice, Gregory T. Wann, and Justan L. Blair

The Current River is home to approximately 10 species of aquatic turtles. This river is supplied with spring water throughout it's course. The temperature of the water coming from these springs is much colder than the temperature of the river itself. The mixing of the spring water with the river causes the temperatures downstream of these springs to be colder than those upstream. Our project looked at the effects of these temperature changes on aquatic turtle abundance. We would expect that due to these turtles being exothermic they would prefer the warmer water temperatures above the springs over the colder ones downstream from the springs. This would suggest that there would be more turtles found above the springs, than below. For our study, we used the areas upstream and downstream of Round Spring, Alley Spring, and Blue Spring. For each spring, the river 400 meters above and 400 meters below was used. During each run water temperature was taken and observed turtles were counted. Three trials were used for each spring in order to calculate the number of turtles above and below the springs.

Debora Rivera

Hometown: Jersey City, New Jersey

Major: Psychology and Sociology

University: The College of Saint Elizabeth

Faculty Mentor: Dr. Lisa Flores, Educational & Counseling Psychology

Funded by Louis Stokes Missouri Alliance for Minority Participation

Research using social cognitive career theory with diverse racial and ethnic groups: An overview and meta-analysis

Debora Rivera and Lisa Flores

The present study reports on the meta-analytic findings connected with two proposed pathways in Lent, Brown, & Hackettís (1994) Social Cognitive Career Theory: the pathway from background contextual affordances to self-efficacy and the pathway from self-efficacy to interests. More precisely, this study uses meta-analysis to assess the relations of acculturation and interests to self-efficacy for U.S. racial and ethnic minorities. Three and five of research studies were analyzed for the background contextual affordances-self-efficacy and self-efficacy-interests pathways, respectively. Results for the meta-analysis are still pending. Implications for this study suggest a need for more extensive research on the applicability of SCCT for culturally diverse populations. In addition, this research emphasizes the need for more career programs that attend to issues of acculturation, self-efficacy, and interests to help facilitate the career development of individuals from culturally diverse backgrounds.

Aida N. Ruiz

Hometown: Caguas, Puerto Rico

Major: Chemistry

University: Universidad del Turabo

Faculty Mentor: Dr. John J. Tanner, Chemistry

Funded by Louis Stokes Missouri Alliance for Minority Participation

An Introduction to protein crystallization and x-ray diffraction

Aida N. Ruiz Ruiz and John J. Tanner

Our ability to describe and utilize protein structure and to define interactions with ligands has made possible the rational design of new drugs and pharmacological agents. (Alexander McPHERSON, Current approaches to macromolecular crystallization). The major experimental technique for obtaining the three-dimensional structures of proteins is X-ray crystallography, which involves four steps: (1) protein expression and purification, (2) crystallization, (3) X-ray diffraction data collection, and (4) computational analysis. This summer, I am learning the tools and techniques of steps 2 and 3. First I started learning the procedures and how to grow crystals with a test protein called lysozyme. Two methods for growing crystals where investigated: sitting drops and hanging drops. Both methods yielded large crystals. Next, I prepared lysozyme protein crystals for X-ray data collection by scooping them up in a small loop and plunging them into liquid nitrogen. The frozen crystals were transferred to the X-ray diffraction instrument and a diffraction data set was recorded. These crystal growth techniques were then applied to the crystallization of an anti-single-stranded DNA antibody fragment. Such antibodies are important because of their involvement in autoimmune diseases such as systemic lupus erythematosus. Crystallization of the antibody fragment without and with and an oligodeoxythymidine ligand was attempted. This poster will present the latest results of these crystallization experiments

Barbara Sanchez Neri

Hometown: Elko, Nevada

Major: Biochemistry

University: Purdue University

Faculty Mentor: Dr. James Schoelz, Plant Microbiology & Pathology

Funded by Plant Genomics Internship at MU

Characterization of virus resisatnce in *Nicotiana*

Barbara Sanchez Neri, John Cawly, Jillian Lane, and James Schoelz

Plants are able to defend themselves from pathogen attack because of resistance genes. Resistance genes can recognize specific proteins of a pathogen, resulting in the activation of host defenses. One plant that may contain as many as 70 virus resistance genes is *N. glutinosa*. For example *N. glutinosa* is the source of the N gene, which specifies resistance to *Tobacco mosaic virus* (TMV). I contributed to two projects. One project involved the introgression of the chromosome that contains the N gene from *N. glutinosa* to *N. clevelandii*. The incorporation of this chromosome into the *N. clevelandii* genome should allow us to identify other virus resistance genes located on that chromosome. The other project involves the characterization of the N gene from *N. edwarsdonii*, a Nicotiana species that contains the N gene but is susceptible to TMV. The immediate goal of this project is to determine if the N gene nucleotide sequence might contain a mutation that undermines its effectiveness in preventing TMV infections.

The first project involved the evaluation of crosses between *N. glutinosa* and *N. clevelandii* for the presence of the N gene. The progeny were backcrossed to *N. glutinosa* for three generations and than that generation was selfed. Resistance or susceptibility was tested by inoculating the plants with TMV. A hypersensitive response, the development of necrotic lesions, indicated that the plant was resistant. Yellowing of the leaves and eventual death of the plant confer susceptibility. The second project involved making primers for specific 1000 base pair regions of the N gene. The primer product was than gel isolated, cleaved with restriction digest enzymes, and ligated into the plasmid pGEM7zf. This ligation mixture was then transformed into E. coli grown on LB plates, and colonies purified. The purified product was then sequenced. The sequence can than be compared to the original N gene. Currently there are no results; therefore no conclusions can be drawn as of now.

Scott J. Schoenleber

Hometown: Columbia, Missouri

Major: Biology and Spanish

University: University of Missouri-Columbia

Faculty Mentor: Dr. Habib Zaghouani, Molecular Microbiology & Immunology

Funded by Arts and Science Undergraduate Research Mentor Program

Glutamic acid decarboxylase 65 peptide (206-220) expressed on an immunoglobulin chimera provides differential protection from diabetes onset prior to and following insulin autoantibody seroconversion

Scott J. Schoenleber, Randal K. Gregg, J. Jeremiah Bell, Hyun-Hee Lee and Habib Zaghouani

Glutamic acid decarboxylase isoform 65 206-220 aa sequence (GAD2) was expressed on an Ig molecule and the resulting Ig-GAD2 chimera was tested for peptide presentation and suppression of diabetes in non-obese diabetic (NOD) mice. The findings indicate that Ig-GAD2 is much more efficient for peptide presentation than are free GAD2 peptides. Such effective presentation was able to drive peripheral T cell tolerance as the injection of Ig-GAD2 in saline (non-inflammatory conditions) delayed diabetes development in NOD mice. When administered at the pre-insulitis stage, soluble (sol) Ig-GAD2 only slightly delayed the onset of diabetes, whereas aggregation of the chimera produced a much more significant delay. Initial studies have indicated that this could be due to the upregulation of IL-10 produced by APCs, which, combined with peripheral tolerance, drives a multi-modal suppression of diabetes. To test the suppressive capabilities of agg Ig-GAD2 in a more clinically relevant situation, we injected mice that had seroconverted to produce insulin autoantibodies (IAA), which serve as a marker for diabetes predisposition. Surprisingly, agg Ig-GAD2 was ineffective in suppressing diabetes development in IAA-positive mice, while sol Ig-GAD2 provided substantial protection. Due to the overwhelming number of identified T cell epitopes associated with diabetes progression, single epitope immunotherapies have shown little success. However, delivery of the GAD2 epitope by the Ig-GAD2 vehicle holds promise for successful antigen-specific interventions aimed at preventing the spontaneous development of diabetes in the NOD mouse.

Jasmine V. Scott

Hometown: Jackson, Mississippi

Major: Animal Sciences

University: Alcorn State University

Faculty Mentor: Dr. David Ledoux, Animal Sciences

Funded by Louis Stokes Missouri Alliance for Minority Participation

Re-evaluation of the phosphorus and calcium requirements of tom turkeys from hatch to three weeks of age

Jasmine V. Scott, J.N. Broomhead, and D.R. Ledoux

This study re-evaluates the Ca and P requirements of tom turkeys from hatch to three weeks of age. Calcium and P are extremely important in the development of young turkeys. They are essential for bone formation, skeletal growth, egg production, shell quality, and other metabolic functions. The purpose of this study is to find a diet providing adequate amounts of Ca and P that satisfies but not exceeds nutritional requirements. Though P is important, too much consumption will result in large amounts of P in the excreta, which is a waste of money and is harmful to the environment.

A 3x4 factorial design was used with 3 levels of Ca [1.0, 1.2, &1.4%] and 4 levels of P [.40, .50, .60, & .70%], making up 12 diets. Within each diet, there were 6 replicate pens of 15 turkeys. The National Research Council (NRC) recommends 1.2% Ca and 0.60% P. However, some diets contained more than the NRC standard and some contained less. After 21 days (3 weeks) of age, the turkeys were weighed and their feed consumption was determined. They were euthanized with Carbon Dioxide and their tibias were collected for bone ash and bone breaking strength tests. The results showed that the turkeys that were fed 0.5% 0.6%, and 0.7% P had stronger bones and better performance than those who were fed 0.4%. Calcium did not affect the performance of bone strength.

Marcos Searight

Hometown: Biloxi, Mississippi

Major: Psychology

University: Jackson State University

Faculty Mentor: Dr. Michael Mobley, Educational & Counseling Psychology

Funded by Louis Stokes Missouri Alliance for Minority Participation

Hopes and goals among African- American adolescent males

Marcos Searight and Michael Mobley

This research study will use qualitative techniques to investigate African-American males hopes and goals to understand the influences that facilitate the achievement on their hopes and goals. This study addresses the question, *How do social factors influence African-American adolescents males to become who they are in society?* These influences may support or hinder African-American males from fulfilling their hopes and goals. Potential social influences may include the media, the community, and the educational system, each of which may, portray different messages to African-American adolescents males regarding their ability to achieve their hopes and goals. The results will be presented and implications will be discussed.

Kyle M. Shull

Hometown: Higginsville, Missouri

Major: Biology

University: Southwest Missouri State University

Faculty Mentor: Dr. Carl Gerhardt, Biological Sciences

Funded by National Science Foundation - REU (Life Sciences)

Effect of predator chemical cues experienced during the egg stage on larval development of the grey treefrog, *Hyla versicolor*

Kyle M. Shull, Michael J. Smith, and H. Carl Gerhardt

Developmental plasticity may be adaptive in situations where behavioral responses to predation are limited, such as during the egg stage. Studies in the past have shown that the developmental rates in frogs can be influenced by predation on the eggs; the presence of predators that prey on the eggs themselves may cause an increase in the developmental rate during the egg phase, while predators present that prey on tadpoles cause a decrease in the developmental rate. All previous studies, however, included the predator with the eggs. These fail to distinguish between differences due to chemical cues and differences due to behavioral cues. In our experiment, we tested the effects of only chemical cues on the grey treefrog, *Hyla versicolor*. We exposed eggs (n=30 per treatment) to water (5mL per day for 4 days) from containers that held individual predators from one of four treatments (crawfish, leech, dragonfly larvae, or no predator [control]). Thirty days after fertilization, data show that tadpoles exposed to the leech treatment were significantly smaller that those of the other treatments. Body size and developing rate measurements will continue to be made up to and beyond metamorphosis.

Annie Silkowski

Hometown: Bozrah, Connecticut

Major: Biology

University: University of Connecticut

Faculty Mentor: Dr. Paul Porneluzi, Dr. John Faaborg, and Rick Clawson, Biological Sciences

Funded by Missouri Ozark Forest Ecosystem Project

Effect of forest management techniques on species richness and abundance of ground layer herbs

Annie Silkowski, Paul Hage, and Scott Loss

We compared species richness and abundance of ground layer herbaceous plants (excluding grasses) on sites with different forestry management techniques. The three sites studied were: even-aged (10-15% of timber harvested in several clearcuts that averaged 3-13 ha. in size), uneven-aged (10-15% of timber harvested in many small circular cuts 21-43 m. in diameter), and a control (no timber harvested). Ten random points on each site were picked where we used one-square-meter quadrats to collect data. Focus was placed on three species: Hogpeanut (*Amphicarpa bracteata*), Virginia Creeper (*Parthenocissus quinquefolia*), and Blackberry (*Rubus allegheniensis*). Species richness and abundances were determined and compared between the sites. These characteristics of plants may be a good predictor of overall species diversity and ecosystem health. This study was undertaken in the summer of 2003 in the Current River Conservation Area near Ellington, Missouri.

Antonia Sisneros

Hometown: Mora, New Mexico

Major: Biological Engineering

University: University of Missouri-Columbia

Faculty Mentor: Dr. Kevin Gillis, Dalton Cardiovascular Research Center

Funded by the McNair Scholars Program

Determining the sensitivity of Ca^{2+} sensors to Ca^{2+} surrogates: Creating a calibration curve

Antonia Sisneros and Kevin Gillis

The calcium ion (Ca^{2+}) triggers the release of neurotransmitter and hormones from neurons and neuroendocrine cells through a process called exocytosis. It is important to understand the nature of these sensors because they are vital to the function of the nervous and endocrine systems.

Although various methods have been used to study the process of exocytosis, the identity of the Ca^{2+} sensors that trigger exocytosis is still unknown. It is known that the Ca^{2+} sensors that trigger exocytosis are sensitive to several ions of similar size and charge as Ca^{2+}, but the sensitivity varies among ions. Looking at how various Ca^{2+} 'surrogate' ions stimulate exocytosis will indicate the size, flexibility, and accessibility of the Ca^{2+} binding sites on exocytosis related proteins and, therefore, point toward the identity of the proteins.

In this study we focused on the Ca^{2+} surrogate strontium (Sr^{2+}). In order to measure the concentration of Sr^{2+} inside the cell we used the indicator dye, fura2-ff, which is normally used to indicate Ca^{2+} concentrations. Fura2-ff is a ratiometric dye, which means that it changes its excitation spectrum when it binds Sr^{2+} or Ca^{2+}. We calibrated the dye by measuring the ratio of fluorescence excited by two wavelengths, 340 nm and 365 nm, and correlating this value with known concentrations of Sr^{2+}.

We used a calibration solution made of $SrCl_2$ mixed with the Sr^{2+} chelator (binding molecule) EGTA. At a given pH and total concentration of Sr^{2+} and EGTA there is an equilibrium established between Sr^{2+} bound to EGTA and free Sr^{2+} creating a stable concentration.

We fit the Grynkiwicz equation, $[Sr^{2+}] = K_{eff} * (R - R_{min})/(R_{max} - R)$, to the data from four different Sr^{2+} concentrations. This equation is used to describe the relationship between the ratio of wavelengths (R) and the concentration of Sr^{2+} ($[Sr^{2+}]$). R_{max}, R_{min}, and K_{eff} are constants that were obtained when the equation was fitted to the collected data. The resulting equation will be used to find an unknown Sr^{2+} concentration from an observed fluorescence ratio.

Tatiana Sousa

Hometown: Elizabeth, New Jersey

Major: Biology

University: The College of St. Elizabeth

Faculty Mentor: Dr. Miriam Golomb, Biological Sciences

Funded by National Science Foundation - REU (Life Sciences)

Epitope tagging of a candidate virulence protein of *Haemophilus influenzae*

Tatiana Sousa and Miriam Golomb

Haemophilus influenzae(HI) is a commensal bacterium of the human nose and throat. HI can also cause diseases ranging from infections of the ear to meningitis. Nonencapsulated HI strains can also be invasive and cause diseases. These strains are resistant to vaccination. We are investigating the virulent HI strain R2866, which has a potential virulence factor, the *lav* gene. Lav, the inferred protein product, belongs to the autotransporter family of virulence proteins. Autotransporters are outer membrane proteins that form a pore with their carboxyl domains, through which they export their N-terminal œpassenger" domains.

To investigate the function of Lav, we need specific antibodies. My project is to place an epitope tag in the *lav* gene of R2866 in a site that will be exposed on the surface of the bacterial cell and allow normal function of the protein.

The FLAG tag was inserted into a predicted external loop of the carboxyl domain. This is done in two steps. First, the left and right-hand segments of the gene were amplified by PCR; the FLAG sequence was built into the forward primer for the right-hand segment. The two segments were cloned into an *E. coli* plasmid in sequence, linked in frame by a restriction site (introduced in the PCR primers). Finally, we will insert a selective antibiotic resistance cassette just 3' to *lav*; this cassette contains Haemophilus-specific uptake sequences. This construct will be transformed into the R2866 chromosome, replacing its *lav* gene by homologous recombination. Ribostamycin-resistant transformants will be checked for presence of the modified *lav* gene and expression of the FLAG tag

Epitope tagging of the *lav* gene will assist in tracking the Lav protein's location, expression and proximity to host cells.

Patrick D. Stanley

Hometown: Columbia, Missouri

Major: Biology

University: University of Missouri-Columbia

Faculty Mentor: Dr. Paul Porneluzi, Dr. John Faaborg, and Rick Clawson, Biological Sciences

Funded by Missouri Ozark Forest Ecosystem Project

Movement of worm-eating warblers (*Helmintheros vermivorus*) and black-and-white warblers (*Mniotilta varia*) from mature forest to clearcuts

Patrick Stanley, Joseph Patson, and Merande Green

During MOFEP 2003, in Reynolds and Shannon County, Missouri, we studied a potential trend in the movement of Worm-eating warblers (*Helmintheros vermivorus*) and Black-and-White Warblers (*Mniotilta varia*) from mature forests to clearcuts. Researchers have noted that these birds may be moving into the clearcuts with their fledged families. This is possibly because the clearcuts are good places for protection from predation and for foraging. Understanding how birds use the land during and after their breeding cycles is critical for the development of land management practices that can preserve them.

We examined spot map data from undisturbed and disturbed forest, as well as from clearcuts to determine if birds were moving from mature forest to clearcuts. We counted the number of observances of the two species within three different two-week periods for the three separate types of landscapes. We then graphed the data for each landscape type to observe any trends. If forest interior birds are using these clearcuts, even-aged management may present some advantages to these species in addition to previously recognized benefits to early successional species.

Melissa M. Steward

Hometown: Florissant, Missouri

Major: Biology Education

University: University of Missouri-Columbia

Faculty Mentor: Dr. Mark. D. Kirk, Biological Sciences

Funded by Life Sciences Undergraduate Research Opportunity Program - Academic Year

Astrocyte-derived factors induce the expression of neural markers in human mesenchymal stem cells

Melissa M. Steward, Jason S. Meyer, Kristy D. Wendt, and Mark D. Kirk

Mesenchymal stem cells (MSCs) are non-hematopoietic adult precursor cells found in bone marrow. Previous studies have shown that these cells can differentiate into neuron-like and or glial-like cells. Since they can be derived from bone-marrow and can differentiate into neuron-like or glial-like cells, MSCs are a valuable potential source of autologous stem cells for treatment of CNS disorders.

In the present study, we tested an experimental protocol for it's ability to neuralize MSCs *in vitro*. (i.e. induce MSCs to become neural cells). Human MSCs (hMSCs, provided by Neuronyx, Inc.) were cultured up to 12 days in medium conditioned by rat astrocytes. Previous studies had indicated that co-culture with rat astrocytes qualitatively increased the percentage of neuron-like cells. The hMSCs in the current study were subsequently labeled using immunocytochemistry to determine if the hMSCs were induced to express neuronal markers. The percentage of cells positive for neuronal markers was quantified, as well as the percentage of cells with primary process outgrowth.

Prelimary data suggests that diffusible factors (and potentially cell surface proteins) produced by astrocytes can induce hMSCs to express neuronal markers *in vitro*. Our ultimate goal is to use hMSCs to alleviate neurodegeneration by directing them towards a neuronal fate and transplanting them into the CNS, thus replacing lost neural cells. Alternatively, the hMSCs may be engineered to secrete neurotrophic factors necessary to prevent cell death within affected regions of the diseased or injured CNS.

Richard Steward, III

Hometown: Pine Bluff, Arkansas

Major: Biology

University: University of Arkansas-Pine Bluff

Faculty Mentor: Dr. Dennis Lubahn, Biochemistry/Child Health

Funded by National Science Foundation - REU (Life Sciences)

Is estrogen regulation of cancer protected genes dependent on NRF2?

Richard Steward, III, Peter Ansell, Jinghua Liu, and Dennis Lubahn

Understanding the ability how estrogens impact the regulation of phase II detoxification enzymes is a key component to understanding why estrogen exposure is a risk factor for the induction of certain cancers. The typical model of phase II enzyme regulation shows that the transcription factor NRF2 dissociates from Keap I in times of oxidative stress, translocates into the nucleus, and binds to the antioxidant response element (ARE). NRF2 binding stimulates the transcription of phase II enzymes, which subsequently metabolizes the oxidative stressors, thereby protecting the cell.

We have shown that estradiol bound estrogen receptors can down regulate the expression of phase II enzymes in certain tissues. We hypothesize that this down regulation of phase II enzymes by estrogen could partially help explain some of the estrogen promoting effects. Due to the importance of Estrogen Receptor alpha (ERα)/ARE signaling in regards to cancer formation, we want to characterize the molecular mechanisms by which 17B-estradiol (E2) bound ERα down regulates phase II enzyme expression. Because of the central importance of NRF2 in regulation of ARE mediated gene expression, we hypothesize that ERα is regulating ARE expression through interaction with this protein. The long term goals of my project were to Co-Immunoprecipitate NRF2 and determine if ERα was present in the copmplex by western blot analysis. In order to perform this, we had to optimize the western blotting conditions that would allow us to detect both ERα and NRF2.

Jennifer Stewart

Hometown: Chicago, Illinois

Major: Interdisciplinary Studies

University: University of Missouri-Columbia

Faculty Mentor: Dr. Mark Flinn, Anthropology

Funded by Louis Stokes Missouri Alliance for Minority Participation

The effects of post-natal maternal stress on the growth and development of the infant in a rural Caribbean village

Jennifer Stewart and Mark Flinn

It has been shown in recent literature that infants of depressed, stressed or mentally unstable mothers develop more slowly than infants of mothers without these problems. With depression, for instance, the motherís feelings may impede her ability to adequately respond to and address her infantís needs. We hypothesize that women who faced more stressful life events will have infants/children that are less developmentally advanced than women whose life events were mostly positive. We will be looking at a population of women and children in a rural village in Dominica, in their natural environment, to see if the womanís emotional status affects her infant/childís growth and development. We will talk to women about the way they feel about themselves and their child (ren), and compare that to cortisol (a hormone that indicates stress) levels taken on previous trips. We will also look at health the records of the mothers and children to pinpoint any illnesses and document the growth of the infant/ child. Our study is important because of the worldwide stigma attached to mental health issues. We feel that if we can link the mental health of the mother to the care/well being of the child, then it will provide a way to effectively address mental health issues and ensure proper infant/child growth and development.

Monica A. Stutz

Hometown: Wildwood, Missouri

Major: Biology

University: Truman State University

Faculty Mentor: Dr. Troy Zars, Biological Sciences

Funded by National Science Foundation - REU (Life Sciences)

The role of dopamine in the modulation of walking behavior in *Drosophila*

Monica A. Stutz and Troy Zars

The mushroom bodies in the *Drosophila* brain are necessary for several behaviors, including olfactory classical conditioning as well as the general modulation of walking behavior activity. Clues to the role of neuromodulators in mushroom body function come from the olfactory classical conditioning in which both octopamine and dopamine have complementary functions. Whether octopamine and dopamine similarly affect the mushroom body dependent modulation of walking activity is an open question. This project examined the role of dopamine in the modulation of walking behavior. In order to delete flies of dopamine, they were maintained on sucrose and 3-iodo-tyrosine. After 5 days of the drug treatment, single flies were placed in small cylindrical tubes where their walking was recorded over 4.5 hours. This was done by recording the number of times a fly walked across an infrared sensor in the center of the tube in 10 minute bins. First results indicate a delayed response to the new environment in the dopamine deleted flies. To confirm these results, flies lacking synaptic transmission in dopaminergic cells using a TH GAL4 and tetanus toxin transgene will be similarly tested.

Kevin S. Tan

Hometown: Columbia, Missouri

Major: Biochemistry

University: Cornell University

Faculty Mentor: Dr. Grace Y. Sun and Dr. Sue Yu, Biochemistry

Funded by NIH Grant to G. Sun

Oxidative agents induce the formation of stress fibers in cultured rat astrocytes and microglia

Kevin S. Tan, Sue Yu, and Grace Y. Sun

Cells in the central nervous system (CNS) are highly susceptible to insults due to oxidative stress. Increased oxidative stress is an important landmark of many neurodegenerative diseases including Alzheimerís disease and stroke. H_2O_2 is a natural oxidant compound generated intracellularly through the respiratory chain reaction coupled with superoxide dismutases. Increased H_2O_2 has been shown to alter a number of metabolic pathways leading to altered cell function and cell death. Menadione is a compound that can release free radicals (superoxide) and H_2O_2 intracellularly. Both H_2O_2 and menadione have been used as oxidant agents to investigate oxidative induced changes in cell signaling pathways and cell death mechanisms. Astrocytes and microglial cells are important cells in the CNS. Besides being immune active, astrocytes are important in offering structural support and providing nourishment for the neurons in the brain. Microglial cells are like macrophages and can migrate to site of injury to remove cell debris. Both astrocytes and microglial cells become reactive in stroke, a condition known to have increased oxidative stress. In this study, we examined the effects of H_2O_2 and menadione on cell morphology and cytoskeletal changes using cultures containing both astrocytes and microglial cells. Astrocytes and microglia were co-cultured and treated for varying durations (0 to 16 h) and at different concentrations of menadione (0 to 200 μM) and H_2O_2 (0 to 800 μM). Astrocytes, microglia, and F-actin fibers were immunostained with anti-glial fibrillary acidic protein (GFAP), biotin-conjugated mouse anti-rat CD11b antibody, and phalloidin, respectively. Cell viability was quantified using the lactate dehydrogenase (LDH) assay. Fluorescence and light microscopy were used to identify glial cell type and F-actin. Treatment of menadione and H_2O_2 initiated cell death and triggered the formation of stress fibers from both astrocytes and microglia in a time and dose-dependent manner. However, menadione appeared to cause induction of more stress fibers and microglial cells appeared to be more responsive to the oxidative changes induced by menadione. These results clearly demonstrated in glial cells, changes in cell cytoskeleton occur prior to cell death.

Gesulla Toussaint

Hometown: Miami, Florida

Major: Biology

University: Barry University

Faculty Mentor: Dr. Anand Chandrasekhar , Biological Sciences

Funded by National Science Foundation - REU (Life Sciences)

Facial motor neuron migration in cultured zebrafish hindbrain explants

Gesulla Toussaint, Stephanie Bingham, and Anand Chandrasekhar

As the vertebrate nervous system develops, new neurons migrate from their original location to other sites in the nervous system. We are examining mechanisms underlying the migration of branchiomotor neurons in zebrafish embryos. These neurons are found in the hindbrain, and control the movement of fish jaw and gill muscles. A subtype called the facial branchiomotor neurons (FBMNs) migrates from its birth place, rhombomere 4 (r4), to its destination, r6 and r7. The zebrafish FBMBs represent an excellent model system to understand the mechanisms underlying neuronal migration. In previous experiments, FBMN migration has been studied by embedding intact live embryos in agarose. However, due to continued morphogenesis and the presence of the yolk cell, we have encountered problems with movement of specimen and image quality. To address these issues, an explant culture was developed. The yolk cell of 15-17 hpf embryos was removed by the injection of a paralyzing solution, AMP-PNP, a membrane impermeant non-hydrolyzable analog of ATP, followed by mechanical dissection. After yolk removal, the embryos were decapitated. The head fragments were immobilized by embedding in agarose in L-15 defined amphibian growth medium on microscope slides. The head explants were cultured for 12-24 hr and the health of the embryos and FBMN migration were monitored.

Head explants developed at about the same rate as intact embryos, although there was a slight increase in the number of cell death in the hindbrain. Nevertheless, the migration of FBMNs and the formation of the axon tracts occurred normally in explants. These promising results will enable us to optimize the culture condition in order to effectively analyze FBMN migration behavior at high resolution in vivo.

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Funded by University of Missouri Research Board Grant to M. Kirk

Transplanted embryonic stem cells participate in chimeric development of retinal layers

Sathya Vadivelu, Kristy Wendt, Jason Meyer, Marty Katz, and Mark Kirk

Embryonic stem (ES) cells are pluripotent cells capable of incorporating into existing tissue. As pluripotent cells, ES cells may differentiate into cells like those found in an existing host tissue and may potentially function like those host cells. To evaluate the capability of mouse ES cells to survive, differentiate, and integrate into the developing retina, ES cells were induced to become neural-like cells and then were transplanted into the vitreal cavity of neonatal Albino mice. To distinguish grafted cells from host cells, the transplanted cells were expanded from B5 mouse embryonic stem cells genetically modified to express enhanced green fuorescence protein (eGFP). Mice were sacrificed at two and four weeks post-transplantation to determine the progression of retinal incorporation and differentiation. After the mice were sacrificed, their eyes were enucleated and sectioned. immunohistochemistry will be performed on these sections to determine the type and maturity of the transplanted cells.

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Determining polymorphism in SSR (simple sequence repeat) genetic markers for molecular breeding in soybean

Mauricia Victor, Kathleen Navarro, Xiaolei Wu, and Henry Nguyen

Molecular breeding is a useful tool for plant breeders. It is a technique that utilizes genetic markers to ensure the effective and efficient combination of valuable traits in the development of soybean [*Glycine max* (L.) Merr.] cultivars. Molecular genetic markers help breeders overcome certain limitations when making selections. Instead of selecting based on phenotype alone, which can be affected by negative environmental factors, breeders can select individuals based on their genetic composition and be more confident that those individuals have a desired trait.

The goal of this experiment was to determine a set of polymorphic SSR (simple sequence repeat) genetic markers that could be used to screen 55 specified soybean populations for molecular breeding. SSR markers were chosen because of their known high rate of polymorphism in soybean.

In order to determine polymorphism, seeds from all of the soybean cultivars that were used as parental lines in the specified crosses were collected and grown in the greenhouse (54 total). DNA from each cultivar was isolated from young leaf tissue using the CTAB method. This method produces a high concentration of good quality DNA for PCR. The DNA samples were diluted to a uniform concentration, and PCR was run using 10 different SSR markers. The PCR products were separated by gel electrophoresis on a 3% agarose gel stained with ethidium bromide, and scored using an Alpha Imager UV exposure camera.

Ten different SSR markers were tested on the 54 parental soybean cultivars. However, data was collected for only 2 of the markers because due to problems with PCR failure. Marker 1 was polymorphic in 20% of the specified soybean populations and marker 2 was polymorphic in 23.6% of the populations.

In conclusion, it will be necessary to test more SSR markers, and to retest the markers that had PCR failure. Additional polymorphic markers are needed to develop a set for molecular breeding, and markers that have a higher percentage of polymorphism in the specified populations are desired.

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Effect of even-aged and uneven-aged timber management on the abundance of woodpeckers in the Missouri Ozarks

Abigail B. Walker

I studied the effect of even-aged and uneven-aged timber management methods on the abundances of four woodpecker species in the Missouri Ozarks. According to pretreatment data from the Missouri Ozark Forest Ecosystem Project, 22% of Ozark forest birds are cavity nesters. It follows that timber management methods which impact the health and availability of cavity trees and snags could impact bird populations.

Uneven-aged management consists of group openings of varying sizes, single tree harvests, and girdled trees. This method provides the opportunity for forest managers to leave those snags and cavity trees that provide nest and forage habitat for woodpeckers and other cavity nesters but may not be ideal for timber harvest. Even-aged management consists of clearcutting and intermediate cutting.

I censused the territory abundance of Pileated, Red-bellied, Hairy, and Downy woodpeckers to better understand the impact of timber management on these tree dependant birds. I conducted the census by creating spot maps of territorial calling and drumming within the Missouri Ozark Forest Ecosystem Projectís study plots in Reynolds, Carter, and Shannon counties. To create these maps, undergraduate interns walked portions of the 800 to 1,200 acre plots and noted the locations of territorial calls at least eight times per plot between late May and early July. I compared three sites from each management practice to three control sites seven years after the harvest.

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The rivalry process and interstate war: A comparative case study of incremental & punctuated equilibrium models

Eugene Walton with Patrick James

Examining interstate rivalry is very beneficial in understanding interstate war. Within rivalry research there is a growing debate regarding process models. As of late the Punctuated Equilibrium model has been advanced as an alternate thesis to the traditional Instrumentalist model.

Identifying the appropriate model has major implications for conflict studies, and real-world relevance. Once the appropriate model is identified researchers can identify and even manipulate the variables that will affect a more desirable outcome. One such outcome is the termination of dyadic-rivalries.

This project moves forward the debate as to which rivalry process model is most appropriate. In addition it furthers rivalry knowledge and helps build consensus within the field. This is achieved by reviewing process models in the context of an established case (Egypt/Israel 1948-1989). In addition, an especially visible gap in the current research is filled, as qualitative case studies are virtually absent in Rivalry Process Model literature.

The units of analysis were the points of conflict within each dyadic pair. Subsequently, the process models were applied to each rivalry and evaluated using multiple coders. The coders evaluated the relevance of each process model as it pertained to this case. Although evidence was found supporting both types of process model, there is sufficient evidence which suggest a type of hybrid model.

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Effects of water temperature on aquatic turtle populations on the Current River

Gregory T. Wann, Matthew E. Rice, and Justan L. Blair

The Current River is home to approximately 10 species of aquatic turtles. This river is supplied with spring water throughout it's course. The temperature of the water coming from these springs is much colder than the temperature of the river itself. The mixing of the spring water with the river causes the temperatures downstream of these springs to be colder than those upstream. Our project looked at the effects of these temperature changes on aquatic turtle abundance. We would expect that due to these turtles being exothermic they would prefer the warmer water temperatures above the springs over the colder ones downstream from the springs. This would suggest that there would be more turtles found above the springs, than below. For our study, we used the areas upstream and downstream of Round Spring, Alley Spring, and Blue Spring. For each spring, the river 400 meters above and 400 meters below was used. During each run water temperature was taken and observed turtles were counted. Three trials were used for each spring in order to calculate the number of turtles above and below the springs.

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Constructing Mestiza: Ethnic identity formation in mixed race Pilipino Americans

Miriam R. Warren and Joan Hermsen

The number of children living in mixed race families more than tripled from 1970 to 1990. The 2000 census was the first time mixed race people had the opportunity to assert identities comprised of multiple races. As the population of mixed race people grows by leaps and bounds, research on this group becomes increasingly important. One area in which more research is needed is ethnic identity formation. Pilipino Americans are the second largest Asian American group. Nearly 14 percent of Pilipino Americans self-identify as mixed race, thus Pilipino Americans are a good case for the study of mixed race people and ethnic identity formation.

This paper addresses Pilipino American identity in two ways. First, it utilizes recently released data from the 2000 U.S. Census to create a demographic profile of Pilipino Americans, and in particular, mixed race Pilipino Americans. This is the first analysis of mixed race Pilipino Americans using the Census 2000 data as such it is groundbreaking. Second, this paper develops a theoretical framework for understanding identity formation in Pilipino Americans of mixed race with particular attention to the structural, familial/community, and individual contributors. This framework may be applied to the identity development of other mixed race people.

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Astrocytes can induce expression of neural markers in human mesenchymal stem cells *in vitro*

Jacqueline Weiss, Melissa Steward, and Mark Kirk

During development it is thought that once a cell has differentiated, it is fate restricted and cannot regress and change its differentiation fate. However, recent evidence suggests that certain adult stem cells are in fact not lineage restricted. For instance, mesenchymal stem cells are derived from the mesoderm and normally become tissues such as bone, cartilage, or fat; however, they can be induced to differentiate into cells of a neuroectodermal origin, an entirely different embryonic germ layer.

This study was conducted to investigate the ability of astrocytes to prompt mesenchymal stem cells to undergo neuronal differentiation. Mesenchymal stem cells were grown either in astrocyte-conditioned medium or on top of killed astrocyte monolayers. Fibroblasts were used as a control for each study. Cultures were then immunostained for either beta-III Tubulin, an early neuronal marker, or neurofilament, a more mature neuronal marker. Results are currently being analyzed by fluorescence microscopy.

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Funded by Life Sciences Undergraduate Research Opportunity Program

Does fiber source and level influence the fiber-degrading microbial population of the horse?

Kim Willer and Monty Kerley

The horse has a digestive system anatomy and physiology that is adapted to a diet rich in fermentable fiber. The health of the horse is dependent upon active fiber fermentation by fiber-degrading bacteria in the colon. My hypothesis is that fiber source and level will influence the number and type of dominant colonic microbes in the horse. Soybean hulls have a high concentration of fiber and are rapidly digestible by anaerobic fibrolytic bacteria that reside in the digestive tract. Soybean hulls have been used in horse feed formulations, however their nutritive value or effect on colonic microbial populations has not been widely studied. The proposed study will determine the nutritional value of soybean hulls and the effect of their level in the diet on the colonic microbial population of the horse. The proposed research will also evaluate timothy hay vs. alfalfa hay from the standpoint of microbial population effects. Four different experimental diets were fed on a four-week cycle. Diets were alfalfa hay, 75% alfalfa hay and 25% soybean hulls, 50% alfalfa hay and 50% soybean hulls, and timothy hay. These diets resulted in variations of fiber level and form. Fecal and blood samples were collected the last week of each cycle. Measurements include fiber digestiblity, and nutrient digestiblity, fiber-degrading microbe enumeration, insulin, blood glucose and blood urea nitrogen. At this time, my laboratory analysis is on going and inconclusive.

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Funded by Life Sciences Undergraduate Research Opportunity Program

Characterizing Babesia genes for candidate vaccine peptides

Rachel Williams, George P. Smith, Melissa A. Nevils, and Leslie J. Matthews

Babesiosis is a tropical cow disease caused by the parasite *Babesia bovis*. This parasite infects the red blood cells in cows in much the same way as the parasite *Plasmodium falciparum* of malaria affects the red blood cells in humans. These parasites also share other characteristics that make Babesiosis a good model disease for malaria. Using a phage-display strategy, certain peptides have previously been selected which could serve as components in a vaccine for Babesiosis; the same technique could be used to find a vaccine for malaria. The main goal of this project is to determine if the selected peptides are "natural" peptides—that is, parts of actual parasite proteins—and if so to gather the available information about the function of those proteins or their homologues in *B. bovis* and other species. To accomplish this, we are probing a *B. bovis* cDNA library with the coding sequences of the selected phage-displayed peptides. Once the complete protein coding sequence is obtained from the cDNA clones, we will determine if the peptide coding sequence is in the same reading frame as the complete cDNA coding sequence. This will verify that the phage displayed peptide is natural. We will then look for homologous proteins for comparison to try to decipher our peptides' function and location in *B. bovis*. So far, all four of the probes I tested have successfully identified several cDNA clones and the corresponding cDNA clones have been selected and purified. The process of discovering their entire DNA sequence is ongoing. This insight will help determine if the peptides will fit the mold of good vaccine components.

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Funded by Life Sciences Undergraduate Research Opportunity Program

Optimizing RNA isolation, reverse transcriptase, and quantitative real time-PCR to determine expression of extracellular matrix proteins in *oim* thoracic aortas

David A. Wirth, Brent J. Pfeiffer, and Charlotte L. Phillips

Primary components of the thoracic aorta critical for tissue integrity are collagen and elastin. Collagen is a rod-like protein that contributes to aortic strength and stiffness, while elastin is a highly extensible protein that contributes to aortic compliance. Type I collagen is the predominate collagen in aortic tissue, normally a heterotrimeric molecule composed on two proα 1(I) chains and one proα 2(I) chain. Our biomechanical and biochemical studies using the osteogenesis imperfecta murine (*oim*) model have shown that the absence of proα 2(I) collagen chain significantly reduces thoracic aortic breaking strength, stiffness, and collagen content. The *oim* model is an exceptional system to study type I collagen's affect on aortic integrity because it is a functional null for the proα 2(I) collagen gene, synthesizing only homotrimeric type I collagen molecules composed of three proα 1(I) chains. The purpose of this study is to determine whether the reduced collagen content in *oim* aortas is regulated by a pre-translational mechanism. My project to date has been to optimize conditions for mRNA isolation, reverse transcription, and quantative real time PCR analysis. Ultimately I will determine the pre-translational expression of the COL1A1, COL1A2, COL3A1, ELASTIN, LYSYL OXIDASE, and TUBULIN mRNA by quantative real time-PCR of thoracic aortas from *oim* , heterozygote and wildtype mice at 3, 8, and 18 months of age.

Total RNA was isolated from aortic tissue using the Qiagen RNAeasy kit and various tissue homogenization techniques were tested such as, a glass homogenizer on ice and a 2-inch and a 4-inch mortar/pestle with liquid nitrogen. We determined the most efficient homogenization method was using the 2-inch mortar/pestle with liquid nitrogen. Reverse transcription (RT) was done on preliminary samples using the Improm-II reverse transcriptase, and PCR was done to test for successful cDNA synthesis of transcripts COL1A1, COL1A2, ELASTIN, and LYSYL OXIDASE. The initial reverse transcription reactions contained a 5mM Magnesium Chloride concentration; however, we determined these reactions did not produce full-length cDNA transcripts when tested via PCR. Thus, the Magnesium Chloride for subsequent reverse transcription reactions was lowered to a 3mM concentration, which effectively produce the long cDNA transcripts needed for the COL1A2, LYSYL OXIDASE, and ELASTIN PCR reactions. Our current work in progress is the real-time RT-PCR analysis determining standards for each transcript to quantify mRNA levels of the amplicons stated above.

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A synthetic lethal screen for Zn transporters in the endoplasmic reticulum of *Saccharomyces cerevisiae*

April Wynn, Colin MacDiarmid, Charissa Ellis, and David Eide

The yeast, *Saccharomyces cerevisiae* has many zinc transporters. In the ER, one of these transporters has been identified as *Msc2*. In addition to this, there are indicators that another Zn transporter exists in the ER. The fact that yeast with a deletion mutation for *msc2* is able to survive is one such indicator. Without Zn in the ER, proteins are often misfolded or not transported properly. In order to identify this other transporter, a synthetic lethal screen was developed to find mutants that had a defect in this additional transporter.

The use of this screen is based on the hypothesis that if there are mutations in both *msc2* and the other transporter, the yeast would have no way to get Zn into the ER and be unable to survive. Therefore, starting with a base strain of yeast that had *msc2*, *ade2* and *ade3* deletions allowed for color to be used as an indicator for the lethal screen. When this base strain is transformed with a plasmid containing *msc2* and *ade3* it is able to grow and exhibits a red color. If the plasmid is lost and the yeast continues to grow it will become white and appear to sector. If the yeast that loses the plasmid dies then the colony will appear solid red because it requires the *msc2* containing plasmid to live. This requirement is due to a mutation in the other ER Zn transporter.

The plasmid-transformed yeast undergoes EMS mutagenesis and then is grown until a visible inspection for sectoring is possible. Then the solid red colonies are identified and streaked out to make sure they are indeed solid red. These colonies are labeled as candidates having a mutation in the other Zn transporter. From here the yeast mutants that grew as solid red colonies have to be further screened to insure that the mutation is not a gene conversion of *ade3* or a mutation that is synthetically lethal with *ade3*. Only red colonies with a mutation in the Zn transporter are desired. Having identified several different mutants with the defect in the other Zn transporter is a starting point to being able to identify what this other Zn transporter in the ER is.

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Fishing for deletion mutants--*glh-4*was caught and *csn-5*likely got away

Christopher Yee and Karen Bennett

All eukaryotes with a germline contain cytoplasmic granules of protein and RNA specific to their germ cells. In the nematode *Caenorhabditis elegans* these germ granules are called P granules. The Bennett laboratory has demonstrated that components of P granules are critical for germline development and fertility. Of particular interest is the germline RNA-helicase (GLH) family, components of P granules, as well as the proteins that interact with the GLH family. So far, knockout mutants have been produced in three of the four *glh*s and in three out of four of their interactors.

The primary objective of this project was to produce gene-knockout mutants for the two remaining genes of interest: *glh-4* and the COP-9 signalosome subunit 5, *csn-5*, a GLH-interactor. A large population of wild type worms was grown, synchronized, and then subjected to mutagenesis with TMP (trimethylpsoralen) and UV-light. Next, progeny of the mutagenized worms were collected and distributed amongst 960 petri plates (approximately 500 worms/plate). After five days of proliferation, samples from each plate were gathered and screened by polymerase chain reaction (PCR) for possible deletion mutations in the chosen genes. After screening ~3 million mutagenized worms, we were successful in producing a csn-5 mutation that upon sequencing is an inversion in the *csn-5* gene; however, this strain has yet to be isolated, perhaps due to the inherent lethality of the chromosomal rearrangement.

However, the *C. elegans* Knockout Consortium in Vancouver recently produced and isolated a *glh-4* mutant, and sent it to the Bennett laboratory for further analysis. This has provided the additional strain we sought. First, the mutation *glh-4 (gk225)* has been characterized by western blot analysis, which has suggested the mutation results in a protein null. The mutant strain is being sequenced to determine the exact point of the deletion. In addition, the new mutant strain requires "backcrossing" against wild type in order to remove other mutations that may have been produced during the mutagenesis. The first of six backcrosses for *glh-4* has been completed with the remaining five to be completed in the fall.

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Loosen up: The effects of DNA methylation and chromatin structure on gene expression in maize

Sarah E. Youngstrom and Karen C. Cone

In eukaryotes, gene expression can be regulated by how tightly the DNA is packaged with proteins to form chromatin in the nucleus. Loosely packed chromatin is accessible to the transcriptional machinery and is readily expressed. In contrast, transcriptionally silenced chromatin is tightly packed and is not accessible for transcription. Tightly packed, transcriptionally silent chromatin is frequently highly methylated on cytosine residues. The relationship of chromatin structure, DNA methylation, and gene expression is not fully understood in plants.

To begin to understand how gene expression is regulated at the level of chromatin, our lab has been involved in a project to construct and analyze transgenic plants carrying dominant negative mutants of putative chromatin genes. One of the mutants is in a gene, *chr106*, which is homologous to an Arabidopsis gene that controls DNA methylation and chromatin structure. We hypothesize that the *chr106* mutation in maize will lead to a decrease in DNA methylation; the effect of this mutation should be evident by analyzing genes that are subject to chromatin level regulation involving DNA methylation.

One such gene is *Pl-Blotched*. This gene is an allele of the maize *Purple* gene, which regulates the production of purple anthocyanin pigments in the maize plant. The *Pl-Blotched* allele is a silenced form of the *Purple* gene, and its pigment phenotype is variegated, rather than solid purple. The expression of *Pl-Blotched* is regulated at the level of chromatin structure and DNA methylation. Compared to normal *Purple*. alleles, *Pl-Blotched* has tighter chromatin and higher levels of DNA methylation. Thus, *Pl-Blotched* could serve as a reporter for the altered *chr106* activity. If *chr106* decreases *Pl-Blotched* DNA methylation and remodels its associated chromatin, then we might expect to get more expression and thus more pigment.

To test this idea, we analyzed backcross progeny of a cross of the *chr106* mutation to *Pl-Blotched*. We used a linked herbicide-resistance marker to follow the dominant *chr106* mutation. We used PCR-based assays to identify *Pl-Blotched* homozygotes. Then, we extracted, measured and compared pigment levels in the herbicide-resistant (*chr106* mutant) plants and the herbicide-sensitive (normal *chr106*) plants. More pigment in the herbicide-resistant plants would lead us to propose that *chr106* is involved in regulating the expression of *Pl-Blotched*.

This study is important because the results help us understand better the relationship of chromatin structure and DNA methylation to gene expression.